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# METABOLISM OF ORGANIC AND INORGANIC PHOSPHORUS:

## A FEEDING EXPERIMENT USING PHYTIN AND SODIUM PHOSPHATES.

BY

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# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
*Washington, D. C., January 15, 1909.*

Sir: I have the honor to submit for your inspection and approval a report on a phosphorus metabolism experiment conducted by F. C. Cook under the supervision of the Chief of Bureau. The report covers an experiment in rabbit feeding, extending over a period of six months, during which organic and inorganic phosphorus were fed, and includes calcium, magnesium, and total and ether-alcohol soluble phosphorus balances. At the conclusion of the experiment, complete analyses were made of the bodies of the rabbits, also of normal rabbits, which furnish some valuable data. Although the number of experiments is limited, the complete review of the literature bearing on the subject, which is included in this paper, greatly enhances its value and the interest both in this country and abroad in the relative value of the organic and inorganic forms of phosphorus, iron, etc., in the body economy makes the issuance of this contribution on the subject advisable.

I recommend that the manuscript be published as Bulletin 123 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley, *Chief.*

Hon. James Wilson,
*Secretary of Agriculture.*

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# METABOLISM OF ORGANIC AND INORGANIC PHOSPHORUS.

## REVIEW OF THE LITERATURE.

Much work has already been done on phosphorus metabolism, both in regard to the inorganic and organic forms of phosphorus, and many investigations have been recorded showing the advantages of the various organic forms, such as lecithin, glycero-phosphoric acid, phytin, etc. Most of this work has been done abroad, although some has been published in this country, notably the researches of Jordan, Patten, and Hart;[a] Mendel and Underhill;[b] and Le Clerc and Cook.[c] It seemed advisable, therefore, to present a general survey of the contributions previously made on this mooted question.

### PHOSPHORUS COMPOUNDS.

In speaking of phosphorus compounds, Bunge[d] states that certain of them probably should be regarded as essential organic food substances for man; also that in all animal and vegetable tissues, in every cell are found two complex organic compounds which are rich in phosphorus, namely, the lecithins and the nucleins.

According to the recent recommendations of the joint committee of the American Physiological Society and the American Society of Biological Chemists on protein nomenclature, the word "proteid" should be abandoned and the word "protein" should designate that group of substances which consists essentially of combinations of $\alpha$-amino acids and their derivatives.

The conjugated proteins are divided into (*a*) nucleo-proteins, (*b*) glyco-proteins, (*c*) phospho-proteins, (*d*) hemoglobins, (*e*) lecitho-proteins. The nucleo-proteins are compounds of one or more protein molecules with a nucleic acid. The phospho-proteins are compounds of the protein molecule with some, as yet unidentified, phosphorus-containing substance other than a nucleic acid or lecithin. The lecitho-proteins are compounds of the protein molecule with lecithins (lecithans, phosphatids).

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[a] Amer. J. Physiol., 1906, *16* : 268.
[b] Ibid., *17* : 75.
[c] J. Biol. Chem., 1906, *2* : 203.
[d] Physiologic and Pathologic Chemistry, 2d ed., 1902.

## LECITHINS.

The lecithins are ester compounds which may be regarded as having been formed by the union of one molecule of glycerol with two molecules of a fatty acid (stearic acid, palmitic acid, or oleic acid), one molecule of phosphoric acid, and one molecule of cholin, with the loss of four molecules of water. The formula for lecithin is $C_{44}H_{90}NPO_9$. The lecithin radical contains one atom of nitrogen for every atom of phosphorus.

Cholin is an ammonium base, the composition of which is accurately known. When heated it splits into glycol (ethylene alcohol), and trimethylamin. Its synthesis corresponds with this decomposition. Wurtz [a] produced it by the action of ethylene oxid and water on trimethylamin. In the animal kingdom cholin has, up to the present time, been found only in lecithin. It was first obtained by Strecker [b] from the bile, which contains lecithin, and hence was called cholin. Liebreich [c] found it among the products of the decomposition of phosphorus compounds from brain tissue. Diaconow [d] showed that it was a product of the decomposition of lecithin. In the new tissues of plants cholin is found in other combinations as well as in lecithin. At present but little is known about the part which the lecithins play in the vital functions.

An important question is whether the lecithins of the body tissues are produced from the lecithins of the food or by synthesis from other materials such as fat, protein, and phosphoric acid. It has been ascertained from experiments on artificial pancreatic digestion that the lecithins take up water and readily split up into glycerophosphoric acid, fatty acids, and cholin. It is not yet known whether this decomposition is complete in normal digestion, or a portion is absorbed unchanged, and if so, how large a portion; whether only the undecomposed part, when absorbed, can be utilized in the building up of the tissues, or the products of decomposition which are absorbed again become united; or finally whether lecithin may also be formed from other material. The absorption of lecithin or of its products of decomposition is complete, according to Bunge, as he states that neither lecithin nor glycero-phosphoric acid can be found in the feces. More recent work, however, by Long [e] seems to show that the feces sometimes contain lecithin in considerable quantities. The presence of lecithin in milk, eggs, and many other foods indicates that this substance is essential in nutrition.

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[a] Centrbl. med. Wissensch., 1868, *6* : 69, 431.

[b] Ann. Chem. Pharm., 1862, *123* : 353; 1868, *148* : 77.

[c] Ibid., 1865, *134*: 29.

[d] Centrbl. med. Wissensch., 1868, *6* : 97, 434.

[e] J. Amer. Chem. Soc., 1906, *28* : 704; Long and Johnson, ibid., 1499.

## NUCLEO-PROTEINS.

By this name are designated those compound proteins which yield true nucleins on pepsin digestion and which, on cleavage with alkali, yield protein and nucleic acid. The nucleo-proteins seem to be widely distributed in the animal body. They occur chiefly in the cell nuclei, but they also often occur in the protoplasm. They may pass into the animal fluids on the destruction of the cells; hence nucleo-proteins have also been found in blood serum. They may be considered as combinations of a protein nucleus with a side chain which Kossel[a] calls the "prostetic group." This side chain, which contains the phosphorus, yields on the decomposition of many nucleo-proteins, such as that from the yeast cell [b] or from the pancreas,[c] besides nuclein bases, also reducing substances, which form crystalline combinations with phenyl-hydrazin. The nucleo-proteins contain from 0.5 to 1.6 per cent of phosphorus.

The nucleo-proteins split into a nuclein and an albumin radicle and the nuclein radicle is further split into nucleic acid and albumin. The nucleic acids on cleavage yield in addition to the purin bases three simple pyrimidin derivatives, uracil, cytosin, and thymin. In a recent article by Osborne and Heyl [d] it appears that all but one-sixteenth of the nitrogen of nucleic acid probably belongs to guanin, adenin, cytocin, and uracil, of which one molecule of each is present for every four atoms of phosphorus.

It is important to distinguish between the nucleo-proteins and the pseudo nucleo-proteins. The latter bodies are obtained as an insoluble residue on digestion of certain nucleo-albumins or phospho-glyco-proteins with pepsin hydrochloric acid. They contain phosphorus but yield no nuclein bases. Among the pseudo nucleo-proteins may be mentioned phospho-proteins and lecitho-proteins. These substances are often fed in the form of casein or vitellin in metabolism experiments.

## NUCLEINS.

The generic name of nuclein has been bestowed upon a large number of very different organic phosphorus compounds, which are to be found in all animal and vegetable tissues, being especially abundant in the nuclei of cells. The nucleins contain about 5 per cent of phosphorus and are formed by the cleavage of nucleo-protein. The nucleins are acids, and the phosphorus is given off as phosphoric acid on boiling with water, and more rapidly on boiling with alkalies or acids. But the organic substances which are combined with the

[a] Arch. Anat. Physiol., Physiol. Abt., 1893, p. 157.
[b] Ibid., 1891, p. 359.
[c] Hammarsten, Zts. Physiol. Chem., 1894, *19* : 19.
[d] Amer. J. Physiol., 1908, *21* : 157.

phosphoric acid appear to be of varying characters. Most nucleins are protein compounds, although a few do not contain protein. Nucleins appear to occur mostly in the tissues, not in a free state, but as compounds with protein as nucleo-albumins, and perhaps also with lecithin, and the gastric digestion separates them from these bodies.

Whether the nucleins of the body tissues arise from the nucleins of food (in which case they would rank among the number of essential food substances), or whether the nucleins are formed in the body by synthesis, is a question of great importance, about which, as in the case of the mode in which the lecithins originate, very little is known. The extensive observations by Miescher [a] on Rhine salmon seem to show that the nucleins as well as the lecithins arise in the animal body by synthesis.

### PHOSPHO-GLUCO-PROTEINS.

This group includes the phosphorized gluco-proteins. These compound proteins are decomposed by pepsin digestion and split off para- or pseudo-nuclein substances, similar to nucleo-albumins. They differ from the nucleo-albumins in that they yield a reducing substance on boiling with acids, and from the nucleo-proteins in that they do not yield purin bases.

Only two phosphorized gluco-proteins are known at the present time. Ichthulin, which occurs in carp eggs and was studied by Walter,[b] was considered by him as vitellin for a time. In regard to solubilities, ichthulin behaves like a globulin. Walter prepared a reducing substance from the para-nuclein of ichthulin, which gave a crystalline combination with phenylhydrazin. The other phospho-gluco-protein is helico-protein, obtained from the glands of the small snail *Helix pomatia*.

### INORGANIC PHOSPHORUS.

In regard to phosphoric acid Hammarsten [c] states that there seems to be no doubt that its importance lies chiefly in the fact that it takes part in the formation of nucleins and thereby indirectly makes possible the processes of growth and division which are dependent upon the cell nuclei. Loew [d] has shown, by means of cultivation experiments on the alga Spirogyra, that only by supplying phosphates (in this case potassium phosphate was used) was the nutrition of the cell nucleus made possible, and thereby the growth and division of the cells. The cells of the Spirogyra can be kept alive, and indeed produce

[a] Cited in Hammarsten's Textbook of Physiological Chemistry, New York, 1908.
[b] Zts. physiol. Chem., 1891, *15* : 477.
[c] Physiological Chemistry, 2d ed., 1898.
[d] Biol. Centrbl., 1891, *11* : 269.

starch and proteins for some time, without a supply of phosphates, but their growth and propagation suffer. Phosphoric acid is also without doubt of importance in the formation of the lecithins and other organic phosphorus compounds. The inorganic forms of phosphorus occur in the bones and teeth as calcium phosphate and magnesium phosphate.

A small part of the phosphorus of the food is in the form of inorganic salts, as in meat, but is mostly in organic combination, as in milk, eggs, etc., as nucleo-albumin, nucleins, casein, lecithin, and vitellin.

## PHOSPHORUS METABOLISM.

Röhmann[a] and his followers, Marcuse,[b] Steinitz,[c] Leipziger,[d] Zadik,[e] Ehrlich,[f] and Gottstein,[g] favor the organic forms of phosphorus, and the opinion of the majority is that the nucleins, not being easily split by the digestive juices, are absorbed with difficulty; consequently the body builds its organic compounds from the more simple organic phosphorus bodies.

The theory of Salkowski, Umber, and the Breslau school is that the body has not the power to build from phosphorus-free protein and inorganic phosphates the organic phosphorus combinations essential to the life of the cell. Lack of phosphates in the food is without influence on phosphorus retention, and excessive feeding of organic phosphorus causes the retention of more phosphorus than the excessive feeding of inorganic phosphates. The advantages of organic phosphorus over inorganic phosphates during the period of growth is shown by Cronheim and Müller[h] by experiments performed on five infants and a boy. The problem was to determine whether the two forms of organic phosphorus, protein-phosphorus and fat-phosphorus, exert the same influence on the assimilation of phosphorus and nitrogen. The foods used were a casein preparation from skimmed milk and a lecithin preparation from the yolk of eggs. The food containing lecithin appeared to favor the retention of calcium, most of which was found in the bones. Experiments were also made upon five dogs, four weeks old at the beginning of the experiment. The food given consisted of milk, rice, flour, and butter. Three dogs received this diet plus egg yolk, and two received plasmon

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[a] Berlin. klin. Wochenschr., 1898, *35* : 789.
[b] Arch. gesam. Physiol., 1896, *64* : 223.
[c] Ibid., 1898, *72* : 75.
[d] Ibid., 1899, *78* : 402.
[e] Ibid., 1899, *77* : 1.
[f] Stoffwechselversuche. Inaug. Diss., Breslau, 1900.
[g] Ibid., 1901.
[h] Zts. diät. physik. Therapie, 1903, *6* : 25.

together with sodium phosphate. The amount of food given was calculated according to the following formula: $(\sqrt[3]{\text{Body weight}})^2$. One dog which was fed on egg yolk died, the histological section showing that death was due to pneumonia. There was no difference in the appearance of the dogs and all grew equally well. The marrow of the bones of the dogs fed on egg yolk was yellow and richer in fat, while the marrow of the bones of the plasmon-fed dogs was red and richer in blood but poorer in fat. On aging, the red marrow became yellow, proving that the dogs which were fed with egg yolk made more progress.

The same experiment was tried with four guinea pigs and one of those fed on egg yolk died of pneumonia in three months. The pigs so fed also showed fatty livers, which weighed more than the other livers. The increase in weight was greater in the case of the pigs fed on egg yolk than in the case of those which were fed plasmon. In all cases the phosphorus content of the brain was the same.

The general conclusion was that the growth of nitrogenous tissue is facilitated if phosphorus is ingested in the form of egg yolk; that is, in organic form. The daily amount of phosphorus needed by the average man, according to Siven,[a] is from 0.7 to 0.8 gram, and according to Ehrström,[b] from 1 to 2 grams. He states that phosphorus is necessary for the proper nourishment of the bones, nervous system, body proteins and cells, and that the body strives to retain the phosphates more than other salts. Other investigations along this line were carried out by Tigerstedt,[c] Renvall,[d] and Schlossmann.[e]

Slowtzoff,[f] in studying the action of lecithin on metabolism, found a plus nitrogen balance accompanied by a diminished excretion of phosphorus and also of purin bases. Where the nitrogen balance was minus, the case could be otherwise explained.

Loewi[g] investigated the metabolism of nucleins. He experimented on himself and found that a part of the nuclein was split in the intestine, the phosphorus of the split portion going into the feces, while the nitrogen was absorbed. The part not split was nearly all absorbed and consequently the phosphorus remained in organic combination. It is possible by nuclein feeding to bring the body into the same nitrogen and phosphoric-acid relation as exists in the nucleins themselves, since nuclein ingestion increases the retention of nitrogen and slightly increases that of the phosphorus.

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[a] Skand. Arch. Physiol., 1901, *11* : 308.
[b] Ibid., 1903, *14* : 82.
[c] Ibid., 1904, *16* : 67.
[d] Ibid., 1904, *16* : 94.
[e] Arch. Kinderheilk., 1905, *40* : 1.
[f] Beitr. chem. Physiol. Path., 1906, *8* : 370.
[g] Arch. exper. Path. Pharm., 1900, *44* : 1; 1901, *45* : 157.

Jacob and Bergell[a] studied the influence of nuclein food on the blood and metabolism and found that it increases the number of the leucocytes. Brücke[b] conducted experiments to show that the benefit derived from egg yolk was due to lecithin. Danilewsky[c] determined that lecithin had great influence on the growth of young animals. Umikoff[d] at about the same time showed that rats and doves died when fed on a phosphorus-free diet and also when fed on an inorganic phosphorus diet plus egg albumin, and barely lived on a nuclein-phosphorus diet, but thrived when lecithin was fed. Selensky[e] also demonstrated the valuable effects of lecithin. Serono[e] was the first to inject lecithin into a human subject, and the experiment gave favorable results. Danilewsky showed that lecithin increased the number of red blood corpuscles and the hemoglobin; also that the appetite, body weight, and growth increased. Moreover, the resistance of the body to disease was greater after lecithin feeding, and the loss of body weight during hunger was decreased. Wildiers,[f] however, did not get results corroborating Danilewsky.

Desgrez and Zaky[g] experimented with guinea pigs and dogs and good results were obtained for four and one-half months after the lecithin feeding was stopped. In the urine there was more nitrogen but less phosphorus than in the controls. A larger part of the urine nitrogen was excreted as urea in lecithin-fed animals and a more complete destruction of the protein was brought about in these cases.

Gilbert and Fournier,[h] Carrière,[i] Claude and Zaky,[j] and others carried on clinical experiments with lecithin and found a resultant increase in appetite, number of red corpuscles, hemoblasts, and hemoglobin.

Gliken[k] made a study of the lecithin content of young animals born blind and helpless and of birds, eggs, etc. He states that the very young animals show a higher lecithin content than do mature animals; that the lecithin content decreases with the growth and age of the animal, and that the young animals come into the world with a large relative amount of lecithin in their bodies.

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[a] Zts. klin. Med., 1898, *35* : 171.
[b] Vorlesungen über Physiologie, 2d edition, 1875, *1* : 270.
[c] Compt. rend., 1895, *121* : 1167.
[d] Biology of Phosphorus, Diss., St. Petersburg, 1895.
[e] Cited by Gilbert and Fournier, Compt. rend. soc. biol, 1901, *53* : 145.
[f] La cellule, 1900, *17* (2) : 385.
[g] Compt. rend., 1904, *139* : 819.
[h] Compt. rend. soc. biol., 1901, *53* : 145.
[i] Compt. rend., 1901, *133* : 314.
[j] Gaz. hospitaux civils militaires, 1901, No. 113, p. 1084.
[k] Biochem. Zts., 1907–8, *7* : 286.

Nerking[a] studied the lecithin distribution in animal organisms, and quotes the lecithin content of the organs of various animals as varying from 0.55 per cent in the pancreas to 1.5 per cent in the liver. Schulze[b] investigated the lecithin content of various plant seeds, and found from 0.5 to 1.5 per cent. This author also determined the lecithin content of various portions of the bodies of rabbits, from which it appeared that the average lecithin content equaled 0.45 per cent of the living weight of the rabbits. In the case of a hedgehog the average per cent of lecithin was 0.82 per cent of the live weight. A study of the stability of egg and brain lecithins has recently been made by Long[c] and a further study of lecithin emulsions was made by Long and Gephart.[d]

In making determinations of the deposition of lecithin and its content in organisms Franchini[e] found that feeding lecithin to rabbits increased the content of this substance and also of glycero-phosphoric acid in the liver and the muscles, but not in the brain. Lecithin remains in the liver sometimes for fifteen days after its ingestion has been stopped. The feeding causes a slight increase of glycero-phosphoric acid and of formic acid but not of cholin. Most of the ingested lecithin is absorbed, since only a very small increase is noted in the feces.

According to observations made by Merservizky,[f] lecithin forms 15.35 per cent of fresh hens' eggs. After six days the lecithin content diminishes. The lecithin of the yolk is a storehouse of food for the developing germ, and is used in the development of the skeletal phosphoric acid, in the building up of the phosphorus of proteins, and for the liberation of energy, after which the fat radical is oxidized.

According to Küttner,[g] the influence of lecithin on the activity of the digestive ferments varies with different enzyms, having a favorable effect upon the activity of the gastric and pancreatic enzyms, but a retarding effect upon others. How lecithin itself is affected he could not determine.

Koch and Reed,[h] in an article on the relation of the extractive to the protein phosphorus in the *Aspergillus niger*, express the view that protein, or in the case of *Aspergillus niger*, nuclein phosphorus is the most important form of phosphorus for cell life. It is formed at the expense of the other forms of phosphorus, excepting lecithin, and its formation is not diminished even in extreme starvation. In building up the nucleins lecithin probably takes no direct part. When lecithin is metabolized some or all of its phosphoric acid may be built up into nucleins as a matter of economy to the organism. The

[a] Biochem. Zts., 1908, *10* : 193.
[b] Zts. physiol. Chem., 1908, *55* : 338.
[c] J. Amer. Chem. Soc., 1908, *30* : 881.
[d] Ibid., p. 895
[e] Biochem. Zts., 1907, *6* : 210.
[f] Russky Uratch, 1907, No. 9, p. 302.
[g] Zts. physiol. Chem., 1906–7, *50* : 472.
[h] J. Biol. Chem., 1907, *3* : 49.

extractive, water-soluble forms of phosphoric acid are the ones from which the others are built and represent the intermediary steps between the phosphates and the more complex phosphorus combinations.

Kalaroukoff and Terroine[a] studied the influence of lecithin on the action of the pancreatic lipase and found very little, if any, increased activity when lecithin was present.

Scott,[b] in his experiment on phosphorus liberation from nuclein compounds, determined that it is more difficult to cause phosphorus to pass from its nucleic acid combination to an inorganic condition than has been supposed.

Michel[c] investigated the quality of woman's milk and found that the utilization of its nutritive materials by infants is nearly complete. The salts were least utilized, 40 per cent of calcium and 10 per cent of phosphoric acid being rejected in the feces.

Keller[d] studied the metabolism of phosphorus by determining the phosphoric acid in the urine of infants fed with woman's and with cow's milk, and found less phosphoric acid so excreted in the case of the breast-fed children. Whether this was due to a greater excretion in the feces or to a better assimilation of the phosphorus of the mother's milk remains to be determined.

In the experiments carried out by Jordan, Hart, and Patten,[e] it was found in the case of cows fed on a high phytin diet that when the amount of phytin fed was reduced the amount of fat in the milk was reduced, although there was no effect on the total solids and casein. There was also a smaller excretion of urine and a tendency to constipation. In these experiments there was a considerable loss of body phosphorus for days, when the cows were fed on a low phosphorus diet, with no apparent ill effects. The amount of phosphorus in the egesta was affected but little by the changes of the phosphorus fed; the organic phosphorus was the portion affected, if any effects were produced at all.

Some experiments by McCollum and Hart[f] indicate that the liver and blood have the property of cleaving the salts of phytic acid with the production of inorganic phosphoric acid. The wide distribution of inosite in the tissues makes it impossible to say as yet whether it is also a product of this cleavage. These results are in accord with those of Mendel and Underhill,[g] who showed that the intestine is not necessarily involved in the excretion of the metabolic products of phytin in certain animals, and also with the conclusions of Scofone,[h] that the enzyms of the digestive tract do not alter phytin. Exami-

[a] Compt. rend. soc. biol., 1907, *63* : 372.
[b] Brit. Med. J., 1906, (2) p. 1791.
[c] L'obstetrique, Paris, 1897, *2* : 518.
[d] Abs., Chem. Centrbl., 1899, *70* : 54.
[e] Amer. J. Physiol., 1906, *16* : 268.
[f] J. Biol. Chem., 1908, *4* : 497.
[g] Amer. J. Physiol., 1906, *17*: 75.
[h] Abs., Biochem. Centrbl., 1905, *3* : 606.

nations of the action of ptyalin, pepsin, and trypsin have confirmed Scofone's results.

Experiments made with extracts of muscle and kidney did not give results which pointed toward the presence of a phytase in these tissues.

Suzuki and Yoshimura[a] studied the distribution of anhydroxymethylene phosphorus (phytin), and give a method for extracting the compound, which is a calcium or a magnesium salt. In the juice of tubers and fruit more inorganic than organic phosphorus is found.

Suzuki, Yoshimura, and Takaishi[b] made an investigation of the enzym which decomposes anhydroxymethylene diphosphoric acid, and state that when rice bran and water are allowed to stand the organic compound will be decomposed and the amount of soluble inorganic phosphoric acid increased. When boiled this action does not take place. The same change takes place when barley and rape seeds are used. No other enzym will do this.

As opposed to the beneficial results of organic phosphorus Keller[c] got very favorable results from feeding normal milk plus inorganic phosphates.

Kochmann[d] studied the changes in the inorganic constituents in the tissues of rabbits poisoned by phosphorus. He made iron, calcium, magnesium, phosphorus, potassium, and sodium estimations in the liver, heart, muscles, and bones and compared them with similar estimations in normal animals. His conclusions are that a definite effect was produced on phosphorus metabolism and that the use of phosphorus in bone affections and as a stimulant is well founded. Calcium, potassium, and sodium replace one another. The magnesium metabolism is also affected in the cases of phosphorus poisoning, and the excretion of phosphorus and calcium run parallel.

More recent work by Hart and McCollum[e] on feeding inorganic phosphates to growing pigs has been conducted for two years. According to the abstract published by the authors, the results clearly indicate that inorganic phosphates, such as bone ash, finely ground rock phosphate, or precipitated calcium phosphate (a mixture of di- and tri-calcium phosphates) can be used by these animals in connection with rations containing insufficient phosphorus. Young animals of 40 pounds weight, receiving inorganic phosphates, together with other salts as supplementary to a ration very low in mineral constituents, grew to be animals of 280 pounds weight,

[a] Abs., Chem. Centrbl., 1907, *78* : 1636.
[b] Ibid., 1637.
[c] Abs., Zts. diät. physik. Therapie, 1901, *5* : 147.
[d] Arch. gesam. Physiol., 1907, *119* : 417.
[e] Abs., Science, 1908, *28* : 217.

and bore litters of fairly vigorous pigs, which on the same ration completed the cycle back to 80 pounds, while animals on the same ration, without the inorganic phosphates, collapsed in three months, losing weight and the use of their legs. Other important observations made are as follows: (1) Animals on a ration extremely low in phosphorus made as large gains, up to 75 to 100 pounds, as did animals receiving an abundance of this element, but after reaching this point the weight was reduced and collapse followed. (2) When such low phosphorus rations as induced these symptoms were supplemented by inorganic phosphates, no unfavorable results appeared. Animals fed a low phosphorus diet, supplemented by inorganic phosphates, made as vigorous a development as other animals receiving all the phosphorus in the organic form. (3) Determination of calcium and phosphorus in the principal organs and tissues of the animals fed on the low phosphorus ration showed that they maintained their normal body composition. The per cent of ash in the skeleton of pigs on a depleted phosphorus ration was reduced to nearly one-half that of pigs which received a normal ration, or the phosphorus-poor ration plus inorganic phosphates. When the animals were starving for phosphorus they derived it from their bones, but always removed calcium and phosphorus in the proportions found in tricalcium phosphate.

### PHOSPHORUS ELIMINATION.

In studying the absorption and elimination of phosphorus and other substances the influence of the reactions of the gastro-intestinal tract is of great importance, as is the character of the ash of the food ingested. All of the conditions influencing acidity and alkalinity affect the absorption and the path of excretion of phosphorus, calcium, and magnesium salts. In the case of herbivora a large portion of these elements is eliminated in the feces, no absorption having taken place. This is likely to happen when the food ash is alkaline and sufficient calcium and magnesium are present to combine with the phosphoric acid. There is then an excretion of these elements through the intestines as well as through the kidneys, and when alkalis are in excess of acids in the gastro-intestinal tract the elimination through the bowel is likely to exceed that through the urine. In omnivorous animals a larger portion of the phosphorus, calcium, and magnesium, as well as the nitrogen, is eliminated by the kidneys than is the case with herbivorous animals.

The subject of phosphorus elimination has been studied under many pathological conditions, and especially in hunger, in the cases of Cetti,[a] Breihaupt,[b] and others.

[a] Senator and Müller: Virchow's Archiv. Suppl., 1893, *131*: 2.

[b] Müller: Virchow's Archiv, Suppl., 1893, *131*: 52.

A phosphoric-acid diabetes, noted by Teissier,[a] and Ralfe,[b] showed a resulting polyurea where as much as 12 grams of phosphoric acid were eliminated per day by the kidneys. In diseases of the kidneys the activities of these organs in eliminating the phosphates may be considerably diminished. In meningitis, on the contrary, a marked increase in the phosphates eliminated is observed in the urine. The statements in regard to the quantity of phosphates in the urine in rachitis and in osteomalacia are somewhat contradictory. A phosphaturia is described, which is more correctly called an alkalinuria, where the phosphates settle out owing to an alkaline reaction. A pathological phosphaturia is also noted. Sendtner[c] showed that there was an increased calcium excretion in cases of phosphaturia. This condition is due to a perversion of metabolism, but serves to illustrate the close relationship which exists between calcium and phosphoric acid.

Voit[d] found that the feces of starving dogs contained phosphates.

The subject of phosphorus elimination has been quite fully investigated by Paton, Dunlop, and Aitchison.[e] In the case of dogs fed on a vegetable diet a large proportion of the phosphorus of the food is not eliminated in the urine. The same thing is true when the phosphorus (inorganic) is injected subcutaneously.

In the case of goats none of the subcutaneously injected phosphorus is found in the urine, neither is any of the body or food phosphorus found in the urine. During lactation the excretion of phosphorus by the bowel is diminished to meet the requirements of milk formation. In the case of dogs there is a diminished excretion of phosphorus in the urine during lactation. The milk of goats contains a large amount of total phosphorus, but a small percentage of organic combined phosphorus.

On giving a soluble glycero-phosphate of calcium by the mouth no increased excretion of phosphorus was detected in the urine of dogs or in the urine or milk of goats.

The excretion of inorganic constituents in the urine was studied by Cathcart and Fawsitt[f] during a fourteen-day fasting period. The excretion of phosphorus fell off gradually. There was a decreased output of calcium, magnesium, sodium, and potassium. The normal ratio of sodium and potassium is reversed in starvation.

Fitz, Alsberg, and Henderson's[g] determinations of phosphoric acid excretion during experimental acidosis in rabbits are to the

[a] Lyon Médical, 1875, *19*: 307.
[b] Lancet, 1887 (2), p. 1243.
[c] Münch. med. Wochenschr., 1888, *35*: 671.
[d] Hermann's Handbuch der Physiologie, 1881, *6*: 345.
[e] J. Physiol., 1900, *25*: 212.
[f] Ibid., 1907, *36*: 27.
[g] Amer. J. Physiol., 1907, *18*: 113.

effect that feeding hydrochloric acid produces first an increase and then a decrease in the phosphorus ($P_2O_5$) excreted in the urine. The determination favors the view that the body phosphates are concerned with neutralizing the acid and with its removal from the body.

Roos[a] on feeding thyroids to dogs got an increased phosphoric acid excretion and after extirpation of the thyroids found that the elimination of phosphorus was decreased. Phosphorus elimination seems to be regulated, in part at least, by those glands, the relationship being similar to that which probably exists between calcium and phosphoric acid and the ovaries, and that between iron and the spleen.

It is difficult to give a typical urine analysis on account of its variations. The following table may be of some value, though only approximate figures are given for the quantities of the most important inorganic constituents which are eliminated by an average-sized person on a mixed diet in the course of twenty-four hours in a quantity of 1,500 cc:

| | Grams. |
|---|---|
| Sodium chlorid ($NaCl$) | 15.0 |
| Sulphuric acid ($H_2SO_4$) | 2.5 |
| Phosphoric acid ($P_2O_5$) | 2.5 |
| Potash ($K_2O$) | 3.3 |
| Ammonia ($NH_3$) | .7 |
| Magnesia ($MgO$) | .5 |
| Lime ($CaO$) | .3 |
| Remaining inorganic bodies | .2 |
| Total | 25.0 |

Phosphoric acid occurs in acid urines partly as double $MH_2PO_4$, and partly as simple $M_2HPO_4$, both of these phosphates being found in acid urines at the same time. Ott[b] found that on an average 60 per cent of the total phosphoric acid was double, and 40 per cent was simple acid phosphate. The total quantity of phosphoric acid is variable and depends on the kind and the quantity of the food. The average quantity of phosphoric acid eliminated by man is in round numbers 2.5 grams, with a variation of from 1 to 5 grams per twenty-four hours. A small part of the phosphoric acid of the urine originates from the burning of organic compounds such as nuclein, protagon, and lecithin within the organism. The greater part originates from the phosphates of the food, and the quantity of eliminated phosphoric acid is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesium phosphates. If the food contains much lime and magnesium, large quantities of earthy phosphates are eliminated in the excrements; and even though the food contains considerable amounts of phosphoric acid in

[a] Zts. physiol. Chem., 1895-6, *21*: 19. [b] Zts. physiol. Chem., 1886, *10*: 1.

these cases, the quantity of phosphoric acid in the urine is small. Such a condition is found in herbivora, whose urine is habitually poor in phosphates. The extent of the elimination of phosphoric acid by the urine depends not only upon the total quantity of phosphorus in the food, but also on the relative amounts of alkaline earths and the alkali salts in the food. According to Preysz[a] and Klug[b] and Olsavszky, the elimination of phosphoric acid is considerably increased by intense muscular work.

From the transformation of tissues rich in protein or phosphorized nerve substances in the body, an equal relation between the nitrogen and the phosphoric acid in the urine might be expected. Many investigations have been made on this point, but the conditions which affect the elimination of phosphoric acid are not yet sufficiently known to permit any definite conclusions being drawn from the observations thus far made.

Of the various forms of phosphate compounds which appear in the urine the following may be mentioned: Tricalcium phosphate, $Ca_3(PO_4)_2$, which occurs only in alkaline urines; calcium diphosphate ($CaHPO_4+2H_2O$) occurs in neutral or only in very faintly acid urines; ammonium magnesium phosphate, triple phosphate, may separate, of course, from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine which has become ammoniacal through alkaline fermentation; amorphous magnesium triphosphate, $Mg_2(PO_4)_2$, occurs with calcium triphosphate in urine rendered alkaline by a fixed alkali, and crystalline magnesium phosphate ($Mg_2(PO_4)_2+22\ H_2O$) which has been observed in a few cases in human urine, and in horses' urine.

Phosphate calculi may consist of a mixture of the normal phosphate of alkaline earths with triple phosphate. They also are composed of a mixture of earthy phosphate, triple phosphate, and ammonium urate, surrounding a foreign body as a nucleus. Calculi consisting of triple phosphates alone and stones of simple acid calcium phosphate are seldom obtained.

## SALTS IN THE ORGANISM.

The body contains in its tissues and liquids a considerable amount of inorganic material. When any organ is incinerated this material remains as ash. If the bones, which are rich in mineral material, are omitted the average amount of ash in the human body amounts to about 0.1 per cent of its weight. It consists of chlorids, phosphates, sulphates, carbonates, fluorids, silicates of potassium, sodium, calcium,

[a] Maly's Jahres-Ber., 1891, *21*:352.
[b] Arch. gesam. Physiol., 1893, *54*:21.

magnesium, and iron: iodin occurs also, especially in the thyroid tissues. In the liquids of the body the main salts are sodium chlorid, sodium carbonate, sodium phosphate, and potassium and calcium chlorid or phosphate. In considering the organic foodstuffs, their value as sources of energy, as well as their function in constructing tissue, is emphasized. The salts have no importance from the former point of view. Whatever chemical changes they undergo are not attended by the liberation of heat energy—none at least of sufficient importance to be considered. They have, however, most important functions, as they maintain a normal composition and osmotic pressure in the liquids and tissues of the body, and by virtue of their osmotic pressure play an important part in controlling the flow of water to and from the tissues. Moreover, these salts constitute an essential part of the composition of living matter. In some way they are bound up in the structure of the living molecule and are necessary to its normal reactions or irritability. Even the proteins of the body liquids contain definite amounts of ash, and if this ash is removed their properties are seriously altered, as is shown by the fact that native proteins when made practically ash-free lose their property of coagulation by heat. The globulins are precipitated from their solutions when the salts are removed. The special importance of the calcium salts in the coagulation of the blood and the curdling of milk is well known, as is also the peculiar part played by the calcium, potassium, and sodium salts in the rhythmical contractions of the heart muscle and the irritability of muscular and nervous tissues. The special importance of the iron salts for the production of hemoglobin is also well known. The nutritive importance of the salts in the diet has been demonstrated by direct experiment.

Dogs were fed by Forster[a] upon a diet composed of ash-free fats and carbohydrates, and meats which had been extracted with water until the salts had been reduced. The animals were in a dying condition at the end of twenty-six to thirty-six days. It is probable that they would have lived longer if deprived of food entirely, with the exception of water, since the metabolism of the abundant diet provided aided in increasing the loss of salts from the body. Lunin[b] has described experiments which indicate that some at least of our salts must be provided for us in organic combinations such as are found in plant and animal foods. In his experiments he found that mice fared well on a diet of dried cow's milk. If fed, however, on a diet containing the organic but ash-free constituents of milk, namely, sugar, fat, and casein, together with the extracted salts of cow's milk, they died in from twenty to thirty days.

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[a] Arch. Hygiene, 1884, 2:385. [b] Zts. physiol. Chem., 1881, 5:31.

In a recent article on alkali salts in the ash of human and cow's milk, Kastle[a] states that the practices which have for their object the reduction of the amount of fat in cow's milk, or the addition thereto of mineral matter available for neutralizing the acids resulting from the processes of metabolism are based on sound practical experience, the important difference between the two kinds of milk as to mineral constituents being as follows: (1) Human milk contains relatively more of its mineral matter in utilizable form than cow's milk; (2) it can supply the organism of the child with relatively larger amounts of available alkali in proportion to the protein than cow's milk.

The idea is advanced that in the milk of various animals the inorganic constituents are present in the same proportion as in the ash of the young animals. Bunge[b] shows that there is a very close relation between the composition of the ash of young rabbits, dogs, and cats, and that of dog's milk, dog's blood, and dog's blood serum. He also makes the statement that the epithelial cells of the mammary glands select from the blood and give to the milk all the inorganic constituents in the proportion needed by the young animal.

Phosphoric acid is an important constituent of milk. According to the same author woman's milk contains 0.31 to 0.45 gram of phosphoric acid per liter, and cow's milk 1.81 to 1.97 grams. It is an important fact that the food of the young furnishes the phosphoric acid in organic combinations.

Many investigators have shown that the phosphorus of cow's milk is not so well absorbed as that of woman's milk. Stoklasa[c] claims that the lecithin phosphorus content of woman's milk is 0.35 per cent, as compared with 0.5 per cent in cow's milk. Blauberg[d] fed a child on mother's milk and studied the metabolism of the salts contained therein. He compared his results with the results obtained by other investigators and concluded that the constituents of mother's milk seem to be better utilized by the system than the constituents of cow's milk.

No complete analyses of the mineral substances of pure, blood-free muscle substance were found. The ash remaining after burning the muscle (which amounts to about 10 to 15 parts per thousand, calculated on the moist muscle) is acid in reaction. The chief mineral constituents are potassium and phosphoric acid. Next in amount are sodium and magnesium, and lastly calcium, chlorin, and iron oxid. Sulphates only exist as traces in the muscles, but are formed by the burning of the proteins, and, therefore, occur in abundant quantities in the ash. The muscles contain such large quantities of potassium and

[a] Amer. J. Physiol., 1908, *22*: 284.
[b] Zts. Biol., 1874, *10*: 111, 295.
[c] Zts. physiol. Chem., 1895–6, *21*: 79.
[d] Abs., Chem. Centrbl., 1897, *68*: 957.

phosphoric acid that potassium phosphate seems to be unquestionably the predominating salt. Chlorin is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is about double that of calcium. These two bodies, as well as iron, occur only in very small amounts.

Sherman[a] in making a determination of the amount of mineral matter required by the human body, examined twenty American dietaries for ash and compared the amount of mineral matter contained in them with the estimated maintenance requirements as found in metabolism experiments. He concludes that iron and protein run parallel and that calcium and phosphoric acid vary; further that the average diets do not supply a sufficient amount of either calcium or phosphoric acid, and as much attention should be paid to the supply of calcium, phosphoric acid, and iron as to protein. Milk and cheese might be substituted for a part of the meat of the ordinary diet and the use of fruits and vegetables should supply a part of the sugar, starch, and minerals. Several other workers have studied ash-free diets.

## CALCIUM COMPOUNDS.

The metabolism of calcium has been extensively studied. There are two forms of calcium which enter into the composition of our food and drink, the organic form in milk, eggs, plant seeds, etc., and the inorganic form, which consists principally of calcium carbonate, calcium sulphate, and calcium phosphate. Both forms are absorbable, the amount absorbed depending on the food taken simultaneously. Among other factors influencing the calcium absorption may be mentioned sodium chlorid, which increases, and the alkalis, which diminish, the amount of calcium absorbed. As noted before, there exists a close relationship between calcium and phosphoric acid.

According to Bunge[b] and Bertram[c] the calcium in plant food is not so well absorbed as that in animal food. Many foods lack calcium, the daily need of which for the human body is, in the opinion of Oberndörffer,[d] 1.5 grams, while Bunge[e] claims double that amount, or 3 grams per diem. The young need milk rich in calcium. Bunge says calcium forms 0.04 per cent of the blood, while Albu and Neuberg[f] cite experiments showing that calcium forms as much as 0.27 per cent of the blood. In arterio sclerosis, Gazert[g]

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[a] Lake Placid Conf., Home Econ. Proc., 1907, *9* : 114.
[b] Lehrbuch der Physiologie des Menschen, 1901, *2* : 88.
[c] Abs., Chem. Centrbl., 1879, *10* : 526.
[d] Berlin. klin. Wochenschr., 1904, *41* : 1068.
[e] Zts. Biol., 1876, *12* : 191.
[f] Mineral Stoffwechsel, Berlin, 1906.
[g] Deutsch. Arch. klin. Med., 1898, *62* : 390.

found from fifteen to twenty times as much calcium in the blood as in normal health. More calcium is absorbed from natural food than from artificial, and in the latter case more calcium is likely to be excreted by the bowels unaltered than normally. The amount of calcium retained by the tissues and its manner of combination depend both upon the quality of the food and the amount of calcium in it.

With the exception of the importance of the alkaline earths as carbonates, and especially as phosphates, on the physical composition of certain structures, such as the bones and teeth, their physiological importance is nearly unknown. The occurrence of earthy phosphates in all proteins, and their great importance in the passage of the proteins from a soluble to a coagulable state, make it probable that the earthy phosphates play an important part in the organization of the proteins. An insufficient supply of alkali earths in the food raises an interesting question as to the effect of this lack on the bony structure.

### CALCIUM SALTS AND COAGULATION.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently great amount of lime salts. In solutions free from lime salts the casein does not coagulate with rennet, but if lime salts are added it is changed so that the solution (even if the enzym is destroyed by heating) yields a coagulated mass, having the properties of curd.

According to Soxhlet[a] the soluble lime salts are only of essential importance in coagulation, while the calcium phosphate is without importance. According to Courant[b] the calcium casein compound on coagulation may carry down with it, if the solution contains dicalcium phosphate, a part of this as tricalcium phosphate, leaving monocalcium phosphate in the solution. The chemical process which takes place in the rennet coagulation has not been thoroughly investigated.

The fibrin ferment, which was called thrombin by Schmidt,[c] is produced, according to Pekelharing,[d] by the action of soluble calcium salts on a preformed zymogen existing in the noncoagulated plasma. Schmidt admits the presence of such a mother-substance of fibrin ferment in the blood and calls it prothrombin.

Brücke[e] showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation in liquids poor in fibrin ferment has been known for a

[a] Münch. med. Wochenschr., 1893, *40* : 61.
[b] Arch. gesam. Physiol., 1891, *50* : 109.
[c] Zur Blutlehre, Leipzig, 1892.
[d] Zts. physiol. Chem., 1896–7, *22* : 245.
[e] Vorlesungen über Physiologie, 2nd ed., 1875, *1* : 270.

number of years through the researches of Green,[a] Ringer and Sainsbury,[b] and others. The necessity of the lime salts for coagulation was first shown positively by the important investigations of Arthus and Pagès.[c] In regard to the manner in which the lime salts act a conclusion has been reached by Freund,[d] who claims that the separation of the excess of calcium phosphate is the cause of a part of the protein becoming insoluble—that is, a cause for coagulation. Weighty objections to this view can be raised, and it is refuted by Latschenberger[e] and Strauch.[f] According to Pekelharing[g] the process is as follows: The prothrombin is converted into thrombin by the action of the soluble lime salts, and fluids which are in all other respects capable of coagulation, but contain only prothrombin and no thrombin, can therefore be coagulated by the addition of soluble lime salts. Thrombin is a lime combination of prothrombin, and the process of coagulation consists in the thrombin carrying the lime to the fibrinogen, which is converted into the insoluble combination of fibrin and lime. Several important papers have appeared, notably those of Field,[h] Morawitz,[i] and Loeb,[j] dealing with the rôle of calcium in the coagulation of the blood. While the literature on this subject has not been fully covered in this report, its importance demands more than a passing reference in a paper dealing with calcium metabolism. It has been shown by the investigations of Cavazzani[k] that the lime salts are of importance in the coagulation of the muscle-plasma as well as in that of the blood.

The inorganic constituents of the bony structure, the so-called bone earths, which remain after the complete calcination of the organic substance as a white, brittle mass, consist chiefly of calcium and phosphoric acid, but also contain carbon dioxid and, in smaller amounts, magnesium, chlorin, and fluorin. Alkali sulphates and iron, which have been found in bone ash, do not seem to belong to the bone tissue itself, but to the nutritive fluid or other parts of the bones. According to Gabriel[l] potassium and sodium are essential constituents of bone ash. The opinions of investigators differ somewhat as to the manner

[a] J. Physiol., 1887, *8*: 372.
[b] Ibid , 1890, *11*: 369.
[c] Abs., Chem Centrbl., 1891 (1), p. 511.
[d] Ibid., 1889 (1), p. 545.
[e] Abs., Chem. Centrbl. 1890 (1), p. 169.
[f] Blutgerinnungstheorie. Diss., Dorpat, 1889.
[g] Abs. Chem. Centrbl., 1892 (2), p. 335.
[h] Centrbl. Physiol., 1903, *17*: 529.
[i] Deut. Arch. klin. Med., 1903–4, *479*: 1.
[j] Beitr. chem. Physiol. Path., 1903–4, *5*: 191; 1904–5, *6*: 269; Arch. Path. Anat. Physiol., 1903, *173*: 35; 1906, *185*: 160; J. Med. Research, 1903, *10*: 407.
[k] Maly's Jahres-Ber., 1892, *22*: 346.
[l] Zts. physiol. Chem., 1893–4, *18*: 257.

in which the mineral bodies of the bony structure are combined with each other. Chlorin and fluorin are present in the same form as in apatite ($CaFl_2$, $3Ca_3P_2O_8$). If the magnesium, chlorin, and fluorin be eliminated, the last according to Gabriel occurring only as traces, the remaining mineral bodies form the combination $3(Ca_3P_2O_8)CaCO_3$. According to this author the simplest expression for the composition of the ash of the teeth is $(Ca_2(PO_4)_2 + Ca_5HP_3O_{13} + H_2O)$, in which 2 to 3 per cent of the lime is replaced by magnesia, potash, and soda, and 4 to 6 per cent of the phosphoric acid by carbon dioxid, chlorin, and fluorin. Analyses of bone earths have shown that the mineral constituents exist in rather constant proportions, which is nearly the same in different animals. The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other formations, such as the marrow, blood vessels, etc., which they contain. This probably also explains the larger quantity of organic substance in the spongy parts of the bones as compared with the more compact parts. Schrodt [a] has made comparative analyses of different parts of the skeleton of the same animal (dog), and has found an essential difference. The quantity of water in the fresh bones varies from 138 to 438 parts per thousand. The composition of bones at different ages has not been definitely determined, but according to the analyses made by Voit [b] of bones of dogs and by Brubacher [c] of the bones of children it appears that the skeleton becomes poorer in water and richer in ash with increase in age. Grafenberger [d] has found that the bones of rabbits from 6½ to 7½ years old contained only 14 to 17 per cent of water, while the bones of full grown rabbits from 2 to 4 years old contained 20 to 24 per cent. The bones of old rabbits contain more carbon dioxid and less calcium phosphate than do those of young ones.

### CALCIUM METABOLISM.

A great many experiments have been made to determine the change in the bone constituents, for instance, when a ration rich in lime and one deficient in lime is fed, but the results have always been indecisive or contradictory. The attempts to substitute other alkaline earths or clay for the lime of the bones have also given unsatisfactory results. Weiske [e] has shown that when young and still rapidly growing rabbits are fed with oats, which are poor in acid and lime, plus magnesium and strontium carbonate, these substances in part pass into the skeleton, but a physiological replacement of lime by magnesium or strontium is not to be expected. On the administration of

[a] Maly's Jahres-Ber., 1877, *6*: 207.
[b] Zts. Biol., 1880, *16*: 55.
[c] Ibid., 1890, *27*: 517.
[d] Maly s Jahres-Ber., 1891, *21*: 290.
[e] Abs., Chem. Centrbl., 1892 (2), p. 590.

madder the bones of the animal are found to be colored red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism of the bones.

Under pathological conditions, as rachitis and softening of the bones, an ossein has been found which does not give any typical gelatin on boiling with water. Otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relationship between the organic and inorganic substances. Attempts have been made to produce rachitis in animals by the use of foods deficient in lime. From experiments on fully developed animals contradictory results have been obtained. In young, undeveloped animals Voit[a] produced, by lack of lime salts in the food, a change similar to rachitis. In full-grown animals the bones were changed after a long time because of the lack of the lime salts in the food, but did not become soft, only thinner (osteo-porosis). The experiments in which the lime salts were removed from the bones by the addition of lactic acid to the food have led to no positive results (Heitzmann,[b] Heiss,[c] Baginsky[d]). Weiske[e] on the contrary, has shown by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral matter in the bones might be diminished. A few investigators are of the opinion that in rachitis, as in osteomalacia, a solution of the lime salts by means of lactic acid takes place. This was suggested by the fact that Weber and Schmidt[f] found lactic acid in the cyst-like altered bony substance in osteomalacia. Well-known investigators have disputed the possibility of the lime salts being washed from the bones in osteomalacosis by means of lactic acid. The recent investigations of Levy[g] contradict the statement as to the solution of lime salts by lactic acid in osteomalacia. He has found that the normal relationship $6P_2O_5$:10Ca is retained in all parts of the bones in osteomalacia, which would not be the case if the bone earths were dissolved by an acid. The decrease in phosphates occurs in the same quantitative relationship as the carbonate; and, according to Levy, in osteomalacia the exhaustion of the bone takes place by decalcification, in which one molecule of phosphate and calcium after the other is removed.

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[a] Zts. Biol., 1880, *16* : 55.

[b] Maly's Jahres-Ber., 1873, *3* : 229.

[c] Zts. Biol., 1876, *12* : 151.

[d] Virchow's Archiv, 1882, *87* : 301.

[e] Abs., Chem. Centrbl., 1892 (2), p. 590.

[f] Cited from Gorup-Besanez. Lehrbuch der physiologischen Chemie. 3d Edition, Braunschweig, 1874, p. 636.

[g] Zts. physiol. Chem., 1894, *19* : 239.

The relative amounts of calcium and phosphoric acid in the teeth are, according to the analysis of Hoppe-Seyler,[a] about the same as in bone earths.

The importance of calcium for the activity of the nervous system and the muscles has been the subject of study by many investigators. The conclusions drawn are that if the amount of calcium is decreased nervous and muscular irritability will result and, conversely, that an increase of the calcium will diminish the irritability of the nerves and muscles. Ringer proved that the frog's heart can be kept beating for long periods upon a mixture of sodium chlorid, potassium chlorid, and calcium phosphate or chlorid, and he laid especial importance upon the calcium. The calcium ions are present in relatively small quantities in the blood, but they are absolutely necessary to contractility and irritability. When present in quantities above normal or when in proportional excess over the sodium or potassium ions they cause a condition of tonic contraction that has been designated as calcium rigor. The calcium promotes a state of contraction, the sodium and the potassium a state of relaxation.

Tigerstedt in his text-book states that calcium salts favor the contraction of the heart, while potassium salts are important for its relaxation. Calcium favors muscular movements of low forms of animal life—the contractility of both skeletal and smooth muscles. He cites the experiments of Voit,[b] who fed pigeons with food containing no calcium, and found that the bones which were used for movements were normal for calcium, while the sternum and skull bones were brittle and even perforated in places.

Falta and Whitney[c] showed that after extirpation of a dog's pancreas, the calcium elimination was increased, though the nitrogen, phosphoric acid ratio remained unchanged. The excretion of uric acid in these cases was doubled.

The importance of calcium salts for the growing organisms is discussed by Aron and Sebauer.[d] Special attention was given to the calcium content of the bones, brains, nerves, muscles, and blood. Dogs and rabbits were used, half of them being fed on a calcium-poor diet. The young animal requires at least 1.2 per cent of its body weight of calcium; a diet supplying a smaller amount is called a calcium-poor diet. Under such conditions nervous and other disorders follow, a condition like rickets being established after continued feeding of such a diet; in these cases the bones contain more water than is normal, that is, a water-rich bone is developed whose

[a] Hammarsten, Textbook of Physiological Chemistry, New York, 1908, p. 449.

[b] Zts. Biol., 1880, *16*: 55.

[c] Beitr. chem. Physiol. Path., 1908, *11*: 224.

[d] Biochem. Zts., 1908, *8*: 1.

organic framework is poor in calcium. The calcium content of the flesh and blood shows no variation and the brain but a slight variation from the normal.

Following some experiments made by Sanford and Lusk at the Yale Medical School on new-born pigs, Wilson[a] studied the influence of diet on the growth of young pigs. Three pigs were killed and analyzed at birth and three were reared on a skim-milk diet. To the diet of one pig, lactose was added; to that of the second, dextrose; and the third was given the skim milk without any added substance. The lactose-fed pig thrived best, while the pig fed on skim milk alone showed the least progress after sixteen days. The analyses showed that the pig fed on skim milk used 52 per cent of the calcium in the food for growth; the lactose-fed pig used 70 per cent; and the dextrose-fed pig 64 per cent. The calcium content of the bodies of the pigs at the end of the experiment was 8.29, 8.03, and 8.13 per cent, respectively. Calcium storage evidently depends on the development of the animal rather than on any specific influence of the milk constituents. Herter[b] found striking retardations in the development of the skeleton of older pigs fed on skim milk for many months, but no evidence of rickets was seen.

W. Camerer, jr.,[c] finds that the calcium content of mothers' milk is barely sufficient to cover the needs of the nursing infant if the percentage composition of the five-months-old baby were the same as that of the new-born baby. The percentage of calcium in the new-born pigs above noted averages 9.4 per cent at birth and is 8.15 per cent at the end of two and one-half weeks' feeding. If the pigs fed on lactose and dextrose had contained 9.4 per cent calcium at the end of the two and one-half weeks' test, an almost complete calcium absorption would have taken place.

Patterson[d] states that when an animal is deprived of all inorganic salts in its food profound constitutional disturbances, resulting in death, are produced. The salts of the blood must not only be present in sufficient quantity to bring the osmotic pressure of the blood to a constant value, but they must also be present in certain definite ratios. Every living cell of the body must be washed by a fluid containing salts of certain monovalent and divalent metals in an unvarying ratio, otherwise a disturbance in the intracellular ion-proteins (Loeb)[e] or colloidal salts (Osborne)[f] is produced. Bearing in mind this necessity for a constant ratio between the various salts of the blood, a number of interesting questions are raised by Patterson in regard to the probable effects of depriving an animal, com-

[a] Amer. J. Physiol., 1902, *8*: 197.
[b] J. Exper. Med., 1898, *3*: 293.
[c] Zts. Biol., 1902, *43*: 1.
[d] Bio-Chem. J., 1908, *3*: 39.
[e] Dynamics of Living Matter, New York, 1906.
[f] J. Physiol., 1906, *34*: 84.

pletely or partly, of one particular metal, say calcium. If the proper ratios are not maintained in the blood, then:

(*a*) Is the excretion of calcium checked wholly or partially? During the progress of his research an article appeared by Goitein[a] which disposes of this question by showing that if a rabbit received less than 0.16 grams of calcium per kilo per day in its food, there was a steady loss of calcium from the body. Lehmann[b] and others have shown that in starvation the calcium excreted exceeds the amount of this substance present in the drinking water taken.

(*b*) Are the other salts of the body reduced *pari passu* by increased excretion? This would entail a considerable fall in the total molecular concentration of the blood, and as the living cells of the body and also the red corpuscles are extremely sensitive to osmotic changes this question may also be answered in the negative.

(*c*) Is the deficiency in the food made good by certain tissues of the body giving up a portion of their calcium to the blood and so keeping the proper inorganic balance in this fluid? That this would be the most probable contingency may be inferred from a number of facts. Forster,[c] who was the first to make observations on the effect of insufficient calcium in the food, found that the muscles lost 56 per cent of their calcium content, while the bones also showed a considerable diminution. Voit[d] found that on a calcium-poor diet the bones were more brittle, the skeleton showed a smaller percentage of dry weight than in the normal animal, and that the quantity of calcium in all organs of the body was more or less diminished.

In the experiments in which rabbits were fed on oatmeal and maize meal, a diet which admittedly leads to calcium starvation, the ratio of the calcium of the blood to the total ash of the blood remained the same as that found in the normal animal. That is to say, the blood underwent no loss of calcium relative to the other salts in the time allotted to the experiment—a result which one might anticipate from the immense importance of the salt ratios of the blood. The ratio of calcium to the total mineral matter in the bones was, however, inconstant, and showed fairly wide fluctuations even in the normal animal. The bones can, without doubt, act as storehouses of calcium and possibly of magnesium. That they lose calcium when the animal is placed on a calcium-poor diet has been proved conclusively. Voit's results, however, tend to show that the bones can lose calcium relatively to the other salts, that is, by a selective autolysis. The experiments on his own body metabolism show that calcium can be readily stored during nitrogen retention.

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[a] Arch. gesam. Physiol., 1906, *115*: 118.
[b] Abs., Maly's Jahres-Ber., 1894, *23*: 497.
[c] Maly's Jahres-Ber., 1873, *3*: 251.
[d] Zts. Biol., 1880, *16*: 55.

More interesting, however, are the experiments involving rectal feeding, calcium being stored despite a continuous drainage of nitrogen from the body. In the latter case, as the protein absorbed from the food was insufficient, the muscles and glands must have diminished in bulk, and yet calcium was retained. This fact rather points to the bones as the place where calcium is stored. In the experiments on himself, and in those with rectal feeding, with a fixed diet the urinary calcium varied but slightly, and the variations, such as there were, ran parallel with the total amounts of urine excreted. This result is not remarkable if it is assumed that the kidney, in order to lighten its work against osmotic pressure, allows a fraction of each of the salts of the blood to escape into the urine. The greater the volume of the urine, therefore, the greater the amount of salts eliminated.

The following theories have been published by Albu and Neuberg[a] concerning the cause of rickets:

1. An insufficient amount of calcium in the food.
2. An inadequate absorption of the calcium salts of the food.
3. A disturbance of calcium retention in the bone-building tissues.
4. A disturbance of calcium absorption in bones themselves (Pfaundler).[b]
5. A connection between rickets and blood pressure based on the theory (Stöltzner) [c] that calcium metabolism is regulated by a secretion of the kidney.

Similar theories as to the cause of osteomalacia were enumerated by the same author as follows:

1. A lack of calcium in the food.
2. A lack of calcium absorption from the food.
3. A decreased alkalinity of blood, following an excess of free acids in the blood which dissolve the calcium salts of the bone.
4. A perversion of metabolism, (Fehling),[d] resulting from a diminished activity of the ovaries, which in time affects the calcium balance.
5. Hoennicke[e] classes osteomalacia as a metabolism disease, the phosphorus metabolism being also affected.

In pathological cases the results and opinions are many and diverse in regard to calcium elimination. For example, Beneke[f] found increased calcium elimination in fever, while Senator[g] obtained opposite results. In characteristic bone diseases, osteomalacia and rickets, the same state of affairs is found.

Calcium and magnesium occur in the urine for the most part as phosphates. The quantity of earthy phosphates eliminated daily is

[a] Mineralstoffwechsel, Berlin, 1906.
[b] Münch. med. Wochenschr., 1903, *50* : 1577.
[c] Jahresbuch f. Kinderheilkunde, 1900, *51* : 73.
[d] Arch. Gynaek., 1890–1, *39* : 171; 1894–5, *48* : 472.
[e] Berlin. klin. Wochenschr., 1904, *41* : 1154.
[f] Grundlinien der Pathologie des Stoffwechsels, Berlin, 1874.
[g] Centrbl. med. Wissensch., 1877, *15* : 357.

somewhat more than 1 gram, and of this amount two-thirds is magnesium and one-third calcium phosphate. In acid urines the simple as well as the double acid earthy phosphates are found, and the solubility of the former (among which the calcium salt, $CaHPO_4$, is especially insoluble) is particularly augmented by the presence of double acid alkali phosphates and sodium chlorid in the urine (Ott).[a] The quantity of alkaline earths in the urine depends upon the composition of the food.

### MAGNESIUM COMPOUNDS.

The relative ratio of magnesium to calcium as eliminated by the body is 1 : 8 or 1 : 9, and consists largely of magnesium phosphate, $Mg_3(PO_4)_2$. The amount of magnesium required by the body per day is 0.6 gram. As in the case of iron, though magnesium is necessary to health, but little magnesium is found in the child's food, namely, milk. The need of magnesium in the system has been studied by Bunge.[b] The magnesium balances have been studied by Blauberg,[c] Cronheim and Müller,[d] Bertram,[e] and Renvall,[f] but are not considered as important as the calcium. Moreover, little study has been given to the elimination of magnesium under pathological conditions.

The elimination of phosphoric acid, calcium, and magnesium depends principally on the character of the food and the relative proportion of animal and vegetable food digested.

## A FEEDING EXPERIMENT WITH RABBITS.

### PLAN OF THE EXPERIMENT.

In these experiments four female rabbits were used, the diet containing as little phosphorus as possible. To two of the rabbits organic phosphorus in the form of crude phytin was fed, and to the other two an equivalent amount of phosphorus in the form of sodium phosphates was given.

It was intended to keep these four rabbits on their respective diets for three or four months, in order that they might become accustomed to the added phosphorus and, further, that it might be completely anabolized, and then to mate them and feed the young rabbits on the same kind of food and on phosphorus in the same respective combinations as that fed to the mother rabbits. When the young

[a] Zts. physiol. Chem., 1886, *10* : 1.
[b] Zts. Biol., 1874, *40* : 111, 295.
[c] Ibid., 1900, *40* : 1.
[d] Zts. diät. physik. Therapie, 1902–3, *6* : 25, 92.
[e] Abs., Chem. Centrbl., 1879, *10* : 526.
[f] Skand. Arch. Physiol., 1904, *16* : 94.

rabbits had lived for several weeks on these diets, it was planned to kill them and to examine their bodies in minutest detail for various combinations of nitrogen and phosphorus. The same procedure was to be carried out in the case of the four female rabbits, and in addition, normal rabbits were to be examined as controls. Unfortunately, it proved impossible to obtain young rabbits under these abnormal conditions, that is, living in closely confined quarters (cages) and fed on an artificial diet.

The work was begun early in November, 1907, and concluded the middle of March, 1908. Complete nitrogen [a] and phosphorus balances were determined during a period of nearly five months. Moreover, the inorganic phosphorus was estimated in the urine by the uranium acetate method throughout the entire time. In addition, during the last four weeks, calcium, magnesium, and ether-alcohol soluble phosphorus (lecithin) balances were included to make the study of the phosphorus metabolism more complete.

At the end of the period the rabbits were chloroformed, and the bones, teeth, blood, livers, nerves (including the spinal cord) and brains were analyzed for nitrogen, total phosphoric acid, lecithin-phosphoric acid, calcium, magnesium, water, ash, and ether extract. Two normal female rabbits were chloroformed and the same procedure followed as in the case of the rabbits artificially fed. In all cases post-mortem examinations were made and slides of the various tissues were prepared and histological changes noted.

## PREPARATION OF FOOD.

The food consisted of carrots, gluten, a mixture of starch and sugar, olive oil, and salt solution. The above constituents seemed to furnish a well-rounded ration, supplying sufficient protein, fat, and carbohydrate for the needs of the body. The rabbits to which the inorganic phosphorus salts were fed received daily 5 cc of a standard salt mixture consisting of 450 grams of sugar, 4 grams of calcium chlorid, 15 grams of sodium chlorid, 30 grams of potassium chlorid, and 1 gram of magnesium sulphate, made up to a volume of 2,000 cc and containing 0.0492 gram of phosphoric acid, in the form of disodium hydrogen phosphate and sodium dihydrogen phosphate, per cubic centimeter.

The rabbits to which the organic phosphorus was fed received daily 5 cc of a salt mixture made so as to supply an equivalent amount of the above mineral salts, allowance being made for the presence of calcium, magnesium, potassium, and phosphorus in the phytin. In this way an equal amount of calcium, magnesium, potassium, and

[a] All the nitrogen work was done by the nitrogen laboratory, Mr. T. C. Trescot in charge.

sodium was given to all four rabbits, and the total amount of phosphoric acid fed was practically equalized.

Gluten was selected as a food high in nitrogen but containing little phosphoric acid. The usual method of washing out the starch from coarse flour was employed. The moist gluten was spread out on sheets of tin and dried on the steam bath. After several days of this treatment the samples were sufficiently dried for grinding, and contained from 12 to 13 per cent of nitrogen.

The organic phosphorus was supplied in the form of phytin, a calcium-magnesium-potassium compound of anhydro-oxy-methylene-di-phosphoric acid which was first isolated by Posternak.[a] This was prepared by extracting wheat bran with 0.2 per cent hydrochloric acid, allowing the starch to settle, decanting off the clear liquid, and to this adding a large volume of 95 per cent alcohol. A heavy flocculent precipitate formed. This was allowed to settle and after the clear liquid had been decanted off, the remainder was filtered. The precipitate was then dried at room temperature by blowing air over it by means of an electric fan. In this air-dried condition the phytin contained from 22 to 30 per cent of phosphorus ($P_2O_5$) in organic form. The uranium acetate titration method showed that no inorganic phosphorus was present.

The nature of phytin has been investigated by Patten and Hart,[b] who gave to it the following composition: Calcium, 1.13; magnesium, 5.80; and phosphorus, 16.3 per cent.

Phytin on heating with mineral acids is decomposed into inosite and phosphoric acid. The investigators just quoted claim there is no decomposition of phytin by enzyms and the same conclusion was reached by Mendel and Underhill,[c] who also studied this question. It is claimed that the proteolytic enzyms of the alimentary tract do not alter phytin, but that the alteration is brought about by the intestinal epithelium. The free acid phytin corresponds to the formula $C_6H_8P_2O_9$. The alkali salts are freely soluble in water and the calcium and copper salts are slightly soluble in water, while the barium and strontium salts are but sparingly soluble in water. Phytin has thus far been found in peas, beans, pumpkin seeds, and red and yellow lupines. The carbohydrates of the food were supplied by feeding a mixture consisting of equal portions of cane sugar and cornstarch. The fat used was olive oil.

The food was prepared in the following manner: The carrots were first chopped into small pieces and a portion was mixed with part of the gluten-starch-sugar mixture. To this was added the phytin and 5 cc of the phosphoric-acid-free salt solution in the case of the rabbits

[a] Rev. gen. bot., 1900, *12* : 5.
[b] Amer. Chem. J., 1904, *31* : 564.
[c] Amer. J. Physiol., 1906, *17* : 75.

fed organic phosphorus. This was made into a thick paste and placed on a small tray in one corner of the cage. The same procedure was followed in the case of the rabbits fed inorganic phosphorus, except that instead of phytin the salt solution containing the inorganic phosphorus was mixed with the food. Thus the rabbits were compelled to eat the food containing the phosphorus before the remainder of the food was given to them. The rest of the carrots, sugar and starch mixture, and 2 cc of olive oil were made into a thick paste and given to the rabbits during the afternoon.

## METHODS OF ANALYSIS.

Each rabbit was confined in a suitable wire cage, which allowed the feces and urine to be easily separated.

After establishing a nitrogen equilibrium, the experiments with the rabbits were commenced. The feces were collected at frequent intervals during the day, owing to the fact that the rabbits persistently ate them. The urine and the food residues were collected daily. All of these samples were composited and analyzed; the nitrogen in the food, feces, and urine being determined according to the Gunning [a] method and the phosphoric acid in the food, feces, and urine by Neumann's [b] method. The phosphoric acid in the urine was determined also by the uranium acetate volumetric method. In this way a check on the amount of phosphoric acid in the urine was obtained, and further, this double determination served as an indication of the presence of organic phosphorus.

The methods employed for water and ash, and for calcium and magnesium [c] were those of the Association of Official Agricultural Chemists. From 2 to 3 grams of the foods or feces were ashed; and in the case of the urine, 200 cc were evaporated to dryness in a platinum dish and ashed. The ether-alcohol soluble phosphorus (lecithin) was determined in the following manner:

Transfer one or more grams of the finely ground substance to a 300-cc Erlenmeyer flask; add 30 cc of absolute ether and extract the whole over night. Filter the ether extract through a hardened filter paper into an ordinary Jena flat-bottomed flask of 500-cc capacity and return any particles of the residue found on the filter paper to the Erlenmeyer flask. Then add to the ether extract residue 60 cc of absolute alcohol and boil the solution for three hours, using a reflux condenser. Filter this alcohol extract while hot into the Jena flask containing the ether extract and wash the residue twice with two separate portions of 25 cc each of hot alcohol, adding the washings to the extract. In the combined ether-alcohol filtrate determine the phosphoric acid by the Neumann [b] method.

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Rev., p. 7.
[b] Zts. physiol. Chem., 1902, *37* : 115.
[c] U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Rev., p. 15, 16.

## PRELIMINARY FEEDING PERIOD.

The results recorded in Table I are especially valuable in view of the fact that they cover a period of practically one hundred days. During this time an attempt was made to mate the rabbits, each one being removed from her cage every four days at 5 o'clock and placed in a large box with a male rabbit and allowed to remain there until the next morning at 9 o'clock. This was repeated fifteen or twenty times in each case. Consequently some loss of feces and urine must have resulted, which loss in the course of an experiment extending over one hundred days would be practically uniform in the case of each pair of rabbits. Such a loss would naturally tend to give somewhat larger apparent nitrogen and phosphoric acid balances. The rabbits had always eaten the food provided for them before being removed from their cages.

Throughout this work the rabbits fed organic phosphorus are given the numbers 1 and 2, and those fed inorganic phosphorus the numbers 3 and 4.

The weight of the rabbits remained constant in the case of Nos. 1 and 4, while in the case of No. 2 a slight average loss of weight resulted and No. 3 showed a gain in weight.

TABLE I.—*Nitrogen and phosphorus metabolism—Preliminary period.*

No. 1.—RABBIT FED ORGANIC PHOSPHORUS.

| Date. | Average weight of rabbit. | Nitrogen (N). | | | | Phosphoric acid ($P_2O_5$). | | | | In urine. | | Absorbed material retained. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total ingested. | Total excreted. | | Daily balance. | Total ingested.[a] | Excreted. | | Daily balance. | Nitrogen. | Phosphoric acid. | Nitrogen. | Phosphoric acid. |
| | | | In urine. | In feces. | | | In urine. | In feces. | | | | | |
| *1907–8.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| November 17–23 | 1,647 | 3.34 | 4.22 | 0.40 | −0.18 | 0.66 | 0.95 | 0.28 | −0.08 | ..... | ..... | ..... | ..... |
| November 24–30 | 1,596 | 5.35 | 4.65 | .92 | − .03 | 1.81 | .13 | .90 | .11 | ..... | ..... | ..... | ..... |
| December 1–7 | 1,596 | 8.22 | 4.91 | .83 | .35 | 2.37 | .77 | .76 | .11 | ..... | ..... | ..... | ..... |
| December 8–14 | 1,654 | 9.33 | 5.22 | .39 | .53 | 3.13 | .50 | .49 | .30 | ..... | ..... | ..... | ..... |
| Average | 1,623 | 6.56 | 4.75 | .64 | .17 | 1.99 | .59 | .61 | .11 | 72.40 | 29.65 | ..... | ..... |
| December 15–21 | 1,656 | 10.10 | 4.78 | .60 | .68 | 3.29 | .68 | .69 | .27 | ..... | ..... | ..... | ..... |
| December 22–28 | 1,663 | 10.10 | 6.33 | .60 | .45 | 3.29 | 1.00 | .69 | .22 | ..... | ..... | ..... | ..... |
| December 29–January 4 | 1,677 | 3.54 | 5.26 | .85 | − .36 | 1.74 | 1.12 | .77 | − .02 | ..... | ..... | ..... | ..... |
| January 5–11 | 1,623 | 7.44 | 5.12 | .54 | .25 | 2.79 | .81 | .44 | .21 | ..... | ..... | ..... | ..... |
| January 12–18 | 1,616 | 7.03 | 4.58 | 1.12 | .17 | 2.67 | .63 | 1.95 | .14 | ..... | ..... | ..... | ..... |
| Average | 1,647 | 7.64 | 5.21 | .74 | .24 | 2.76 | .85 | .91 | .16 | 68.19 | 30.79 | ..... | ..... |
| January 19–25 | 1,597 | 9.56 | 6.40 | 1.08 | .29 | 3.27 | .77 | 1.01 | .21 | ..... | ..... | ..... | ..... |
| January 26–February 1 | 1,587 | 10.06 | 6.06 | 1.32 | .38 | 3.24 | 1.04 | 1.05 | .16 | ..... | ..... | ..... | ..... |
| February 2–8 | 1,588 | 10.19 | 7.34 | 1.02 | .26 | 3.27 | 1.11 | 1.02 | .16 | ..... | ..... | ..... | ..... |
| February 9–15 | 1,567 | 10.60 | 8.11 | 1.33 | .16 | 3.23 | 1.80 | 1.16 | .03 | ..... | ..... | ..... | ..... |
| Average | 1,585 | 10.10 | 6.98 | 1.19 | .27 | 3.25 | 1.18 | 1.06 | .14 | 69.11 | 34.71 | ..... | ..... |
| Average for period | 1,618 | 8.08 | 5.65 | .86 | .23 | 2.67 | .87 | .86 | .14 | 69.90 | 31.72 | 22 | 53 |

[a] 1.42 grams of organic phosphorus per period were intimately mixed with the food.

TABLE I.—*Nitrogen and phosphorus metabolism—Preliminary period*—Continued.

No. 2.—RABBIT FED ORGANIC PHOSPHORUS.

| Date. | Average weight of rabbit. | Nitrogen (N). | | | | Phosphoric acid ($P_2O_5$). | | | | In urine. | | Absorbed material retained. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total ingested. | Total excreted. | | Daily balance. | Total ingested.[a] | Excreted. | | Daily balance. | Nitrogen. | Phosphoric acid. | Nitrogen. | Phosphoric acid. |
| | | | In urine. | In feces. | | | In urine. | In feces. | | | | | |
| *1907–8.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| November 17–23 | 1,816 | 4.16 | 4.86 | 0.51 | 0.17 | 1.35 | 0.82 | 0.45 | 0.01 | | | | |
| November 24–30 | 1,728 | 4.32 | 6.09 | .56 | − .33 | 1.98 | .39 | .41 | .16 | | | | |
| December 1–7 | 1,684 | 9.97 | 4.96 | .76 | .60 | 2.88 | .65 | .72 | .21 | | | | |
| December 8–14 | 1,618 | 9.95 | 6.78 | 1.75 | .20 | 3.26 | .66 | 1.35 | .17 | | | | |
| Average | 1,712 | 7.10 | 5.67 | .90 | .16 | 2.37 | .63 | .86 | .14 | 79.86 | 26.58 | | |
| December 15–21 | 1,542 | 11.76 | 7.82 | 2.26 | .23 | 3.37 | .93 | 1.88 | .05 | | | | |
| December 22–28 | 1,480 | 11.76 | 7.84 | 2.26 | .23 | 3.37 | .88 | 1.88 | .08 | | | | |
| December 29–January 4 | 1,505 | 10.67 | 5.85 | 1.19 | .51 | 3.36 | .31 | .95 | .29 | | | | |
| January 5–11 | 1,567 | 10.67 | 5.40 | 1.58 | .52 | 3.36 | .62 | 1.60 | .16 | | | | |
| January 12–18 | 1,646 | 10.67 | 5.61 | .57 | .64 | 3.36 | .41 | .54 | .34 | | | | |
| Average | 1,548 | 11.11 | 6.50 | 1.57 | .43 | 3.36 | .63 | 1.37 | .18 | 58.51 | 18.75 | | |
| January 19–25 | 1,658 | 11.12 | 5.55 | 1.29 | .60 | 3.33 | .83 | 1.24 | .18 | | | | |
| January 26–February 1 | 1,698 | 11.71 | 6.48 | 1.51 | .44 | 3.31 | 1.18 | 1.01 | .15 | | | | |
| February 2–8 | 1,697 | 9.11 | 7.75 | 2.84 | .21 | 2.63 | 1.76 | 1.26 | − .05 | | | | |
| February 9–15 | 1,647 | 8.85 | 6.65 | .83 | .19 | 2.90 | 1.20 | .65 | .14 | | | | |
| Average | 1,675 | 10.20 | 6.61 | 1.62 | .36 | 3.04 | 1.24 | 1.04 | .11 | 64.80 | 40.79 | | |
| Average for period. | 1,645 | 9.47 | 6.26 | 1.39 | .32 | 2.92 | .83 | 1.09 | .14 | 67.72 | 28.71 | 24 | 52 |

No. 3.—RABBIT FED INORGANIC PHOSPHORUS.[b]

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| November 17–23 | 1,474 | 7.53 | 6.48 | 1.20 | −0.02 | 2.83 | 0.58 | 1.02 | 0.16 | | | | |
| November 24–30 | 1,481 | 9.10 | 7.46 | 2.15 | − .07 | 3.27 | .96 | 1.38 | .13 | | | | |
| December 1–7 | 1,447 | 10.40 | 8.89 | 1.12 | .05 | 3.32 | .98 | 1.38 | .13 | | | | |
| December 8–14 | 1,436 | 10.05 | 7.20 | 1.15 | .24 | 3.59 | .99 | 1.15 | .20 | | | | |
| Average | 1,460 | 9.27 | 7.51 | 1.41 | .05 | 3.25 | .88 | 1.24 | .16 | 81.01 | 27.08 | | |
| December 15–21 | 1,401 | 11.70 | 5.48 | .65 | .79 | 3.67 | .84 | .25 | .36 | | | | |
| December 22–28 | 1,484 | 11.70 | 6.14 | .65 | .70 | 3.67 | 1.54 | .25 | .26 | | | | |
| December 29–January 4 | 1,526 | 10.62 | 4.73 | 1.66 | .60 | 3.66 | .63 | .99 | .30 | | | | |
| January 5–11 | 1,598 | 10.62 | 6.50 | 1.50 | .37 | 3.66 | 1.08 | 1.27 | .18 | | | | |
| January 12–18 | 1,652 | 10.62 | 4.89 | .88 | .69 | 3.66 | .82 | .88 | .27 | | | | |
| Average | 1,532 | 11.05 | 5.55 | 1.07 | .63 | 3.66 | .98 | .73 | .27 | 50.23 | 26.78 | | |
| January 19–25 | 1,729 | 11.06 | 5.82 | 1.18 | .57 | 3.64 | 1.06 | 1.17 | .20 | | | | |
| January 26–February 1 | 1,766 | 11.65 | 5.72 | 1.62 | .61 | 3.61 | 1.16 | 1.58 | .12 | | | | |
| February 2–8 | 1,776 | 11.65 | 6.51 | 2.54 | .37 | 3.61 | 1.28 | 1.97 | .04 | | | | |
| February 9–15 | 1,778 | 12.08 | 7.65 | 2.54 | .26 | 3.65 | .85 | 1.98 | .11 | | | | |
| Average | 1,762 | 11.61 | 6.43 | 1.97 | .45 | 3.63 | 1.09 | 1.67 | .12 | 55.38 | 30.02 | | |
| Average for period. | 1,585 | 10.64 | 6.50 | 1.48 | .38 | 3.51 | .98 | 1.37 | .18 | 62.21 | 27.96 | 29 | 54 |

[a] 1.42 grams of organic phosphorus per period were intimately mixed with the food.
[b] 1.72 grams of inorganic phosphorus per period were intimately mixed with the food.

TABLE I.—*Nitrogen and phosphorus metabolism—Preliminary period*—Continued.

No. 4.—RABBIT FED INORGANIC PHOSPHORUS.

| Date. | Average weight of rabbit. | Nitrogen (N). | | | | Phosphoric acid ($P_2O_5$). | | | | In urine. | | Absorbed material retained. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total ingested. | Total excreted. | | Daily balance. | Total ingested.[a] | Excreted. | | Daily balance. | Nitrogen. | Phosphoric acid. | Nitrogen. | Phosphoric acid. |
| | | | In urine. | In feces. | | | In urine. | In feces. | | | | | |
| *1907–8.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| November 17–23 | 1,950 | 8.90 | 8.89 | 0.76 | −0.10 | 3.10 | 1.54 | 0.49 | 0.15 | | | | |
| November 24–30 | 1,960 | 9.14 | 7.91 | 1.13 | .01 | 3.31 | .94 | .94 | .20 | | | | |
| December 1–7 | 1,960 | 10.40 | 4.81 | .42 | .73 | 3.32 | 1.19 | .36 | .25 | | | | |
| December 8–14 | 1,985 | 10.05 | 3.71 | .68 | .80 | 3.59 | .36 | .51 | .38 | | | | |
| Average | 1,964 | 9.62 | 6.33 | .75 | .36 | 3.33 | 1.34 | .58 | .25 | 65.80 | 40.24 | | |
| December 15–21 | 1,965 | 11.70 | 5.16 | 1.06 | .78 | 3.67 | .74 | .90 | .28 | | | | |
| December 22–28 | 2,002 | 11.70 | 8.63 | 1.06 | .32 | 3.67 | 1.42 | .90 | .19 | | | | |
| December 29–January 4 | 1,953 | 10.62 | 8.89 | 1.28 | .06 | 3.66 | 1.44 | 1.42 | .11 | | | | |
| January 5–11 | 1,952 | 10.62 | 6.44 | .26 | .55 | 3.66 | 1.02 | .42 | .31 | | | | |
| January 12–18 | 2,022 | 10.62 | 5.21 | .46 | .70 | 3.66 | 1.04 | .57 | .29 | | | | |
| Average | 1,979 | 11.05 | 6.86 | .82 | .48 | 3.66 | 1.13 | .84 | .24 | 62.08 | 30.88 | | |
| January 19–25 | 2,006 | 7.78 | 5.94 | 1.09 | .10 | 3.20 | 1.55 | 1.09 | .04 | | | | |
| January 26–February 1 | 2,017 | 11.65 | 7.43 | .72 | .50 | 3.61 | 1.29 | .77 | .22 | | | | |
| February 2–8 | 2,000 | 7.06 | 5.44 | .46 | .16 | 2.28 | 1.48 | .54 | .03 | | | | |
| February 9–15 | 1,975 | 8.07 | 6.59 | 2.38 | −.12 | 1.45 | 1.03 | 2.36 | −.13 | | | | |
| Average | 2,000 | 8.64 | 6.35 | 1.16 | .16 | 2.64 | 1.34 | 1.19 | .04 | 73.50 | 50.76 | | |
| Average for period | 1,981 | 9.77 | 6.51 | .91 | .33 | 3.21 | 1.27 | .87 | .18 | 67.12 | 40.63 | 26 | 42 |

[a] 1.72 grams of inorganic phosphorus per period were intimately mixed with the food.

## NITROGEN BALANCES.

The rabbit which gained in weight, No. 3, received a larger amount of nitrogen than the others. In fact, the amount of nitrogen ingested in all cases varied somewhat, but the total during a period of seven days was from 5 to 6.6 grams of nitrogen per 1,000 grams of body weight, No. 3 receiving the largest relative amount. The average figures show that relatively more nitrogen was excreted in the urine in the cases of rabbits No. 1 and No. 2 (those fed organic phosphorus), than in the cases of No. 3 and No. 4 (fed inorganic phosphorus). The amount of nitrogen eliminated in the feces varied with the individual rabbit. No. 1 eliminated 10.6 per cent and No. 2, 14.6 per cent. No. 3 eliminated 13.8 per cent and No. 4 only 9.3 per cent in this manner. Nos. 1 and 2 retained a smaller proportion of the metabolized nitrogen than did Nos. 3 and 4, the figures being respectively 22, 24, 29, and 26 per cent. This means that the rabbits fed inorganic phosphorus retained a larger proportion of the absorbed nitrogen than did those fed organic phosphorus; and it appears that those fed organic phosphorus excreted in the urine a larger proportion of the ingested nitrogen, but did not utilize this nitrogen so well as did the rabbits fed inorganic phosphorus.

PHOSPHORUS BALANCES.

In all cases, excepting rabbit No. 3, the average amount of phosphoric acid ingested during seven days per 1,000 grams of body weight varied from 1.6 to 1.7 grams; in No. 3 the amount was 2.2 grams. More phosphorus was eliminated by rabbit No. 4 through the kidneys than in any other case. Both of the rabbits fed organic phosphorus and No. 3 fed inorganic phosphorus retained about the same amounts of the absorbed phosphorus (averaging 53 per cent), while the figure for No. 4 is much lower, only 42 per cent. From the average figures, it appears that the rabbits fed organic phosphorus eliminated a smaller percentage of the ingested phosphoric acid in the urine than those fed on inorganic phosphorus. It must be remembered that the amount of phosphoric acid eliminated by the kidneys does not necessarily represent the amount metabolized, for inorganic phosphorus ingested might and undoubtedly does pass through the kidneys without undergoing any change. Of the total phosphoric acid ingested, 32 per cent was found in the feces of No. 1 and 31 per cent in the feces of No. 4, while No. 2 and No. 3 eliminated in this manner 37 and 39 per cent, respectively. Although the rabbits fed inorganic phosphorus excreted a larger amount of phosphorus in the urine than did the others, they retained on the average less of the absorbed phosphorus.

The ratio of nitrogen to phosphoric acid in the food is but slightly above 3:1. This shows a much larger proportion of phosphoric acid than is usually fed in a normal diet. The ratio of nitrogen to phosphoric acid in the urine varies from 5:1 to 7.5:1, being higher in the case of the rabbits fed organic phosphorus, owing to the relatively larger elimination of phosphoric acid in the urine of those fed inorganic phosphorus. This ratio in the feces is rather constant, averaging about 1.1:1 in all cases. The exact ratios are given in Table II.

TABLE II.—*Ratio of nitrogen to phosphoric acid in food and excreta—Preliminary period.*

| Rabbit. | Food. | Urine. | Feces. |
|---|---|---|---|
| No. 1 | 3.03:1 | 6.50:1 | 1.00:1 |
| No. 2 | 3.24:1 | 7.54:1 | 1.27:1 |
| No. 3 | 3.03:1 | 6.63:1 | 1.08:1 |
| No. 4 | 3.04:1 | 5.12:1 | 0.91:1 |

## PRINCIPAL FEEDING PERIOD.

The principal feeding experiment extended over a period of four weeks, and during this time complete nitrogen, phosphoric acid, calcium, and magnesium balances were determined, as well as the ether-alcohol soluble phosphorus balance. In addition the inorganic phosphorus in the urine was determined by the uranium-acetate titration method. Other salts, as well as the calcium and magnesium salts,

are important for the welfare of the organism, but in phosphorus-feeding experiments the two named stand out most prominently. The second rabbit fed organic phosphorus was in poor condition during the test and died of pneumonia at the end of the second week; consequently the results in this case must not be given the same weight as in the others. Throughout this period all of the rabbits remained practically constant in weight. Nos. 3 and 4 received more nitrogen and phosphoric acid than did Nos. 1 and 2 on an average, but Nos. 1 and 4 received practically the same amounts. The nitrogen and phosphorus balances were positive, except in the case of rabbit No. 2, which died, and naturally would show a negative set of balances.

## NITROGEN BALANCES.

The analytical data obtained during the experiment are recorded in Table III.

TABLE III.—*Nitrogen and phosphoric acid balances—Principal period.*

RABBITS FED ORGANIC PHOSPHORUS.

| Date. | Rabbit. | | Total ingested. | | Total excreted. | | | | Daily balance. | | In urine. | | Absorbed material retained. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Nitrogen. | | Phosphoric acid. | | | | | | | |
| | No. | Average weight. | Nitrogen. | Phosphoric acid. | In feces. | In urine. | In feces. | In urine. | Nitrogen. | Phosphoric acid. | Nitrogen. | Phosphoric acid. | Nitrogen. | Phosphoric acid. |
| *1908.* | | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *Gms.* | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| February 17–23.... | 1 | 1,565 | 9.891 | [a]2.824 | 0.751 | 6.471 | 0.602 | 0.887 | 0.381 | 0.191 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 1 | 1,551 | 10.176 | [a]3.078 | 2.449 | 6.680 | 1.569 | .970 | .149 | .077 | ..... | ..... | ..... | ..... |
| March 2–8..... | 1 | 1,551 | 10.745 | [a]3.391 | 1.482 | 7.560 | .998 | 1.146 | .243 | .178 | ..... | ..... | ..... | ..... |
| March 9–15..... | 1 | 1,533 | 10.589 | [a]3.483 | 2.310 | 6.470 | 1.461 | .489 | .253 | .218 | ..... | ..... | ..... | ..... |
| Average..... | 1 | 1,550 | 10.3498 | [a]3.194 | 1.748 | 6.795 | 1.157 | .876 | .256 | .166 | 65.65 | 27.41 | 21.0 | 57.1 |
| February 17–23.... | 2 | 1,660 | 7.583 | [a]2.626 | 1.572 | 7.087 | .900 | 1.307 | −.154 | .060 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 2 | 1,623 | 8.519 | [a]2.451 | 3.284 | 7.480 | 1.738 | 1.230 | −.374 | −.085 | ..... | ..... | ..... | ..... |
| Average 2 weeks..... | | 1,642 | 8.051 | [a]2.539 | 2.828 | 7.284 | 1.319 | 1.269 | −.264 | −.013 | 90.47 | 49.97 | ..... | ..... |
| General average..... | | 1,596 | 9.200 | [a]2.867 | 2.288 | 7.040 | 1.238 | 1.073 | ..... | ..... | ..... | ..... | ..... | ..... |

RABBITS FED INORGANIC PHOSPHORUS.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| February 17–23.... | 3 | 1,765 | 12.398 | [b]3.688 | 2.378 | 6.959 | 1.766 | 1.345 | 0.437 | 0.082 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 3 | 1,791 | 12.414 | [b]3.688 | 2.434 | 7.429 | 1.479 | 1.495 | .363 | .102 | ..... | ..... | ..... | ..... |
| March 2–8..... | 3 | 1,814 | 12.149 | [b]3.944 | 2.632 | 7.882 | 1.328 | 1.295 | .365 | .140 | ..... | ..... | ..... | ..... |
| March 9–15..... | 3 | 1,814 | 11.962 | [b]3.944 | 1.133 | 7.882 | .534 | 1.014 | .421 | .342 | ..... | ..... | ..... | ..... |
| Average..... | ..... | 1,796 | 12.231 | [b]3.788 | 2.144 | 7.308 | 1.328 | 1.295 | .397 | .166 | 59.75 | 34.17 | 27.5 | 47.3 |
| February 17–23.... | 4 | 1,986 | 9.157 | [b]3.206 | .596 | 7.680 | .592 | 1.800 | .126 | .116 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 4 | 2,028 | 12.401 | [b]3.688 | .743 | 10.211 | .440 | 1.927 | .207 | .189 | ..... | ..... | ..... | ..... |
| March 2–8..... | 4 | 2,011 | 12.249 | [b]3.933 | 1.198 | 7.520 | .760 | 1.540 | .505 | .219 | ..... | ..... | ..... | ..... |
| March 9–15..... | 4 | 1,971 | 7.72 | [b]3.222 | 1.212 | 7.360 | .728 | 1.806 | −.114 | .098 | ..... | ..... | ..... | ..... |
| Average..... | ..... | 1,994 | 10.395 | [b]3.487 | .937 | 8.193 | .630 | 1.768 | .181 | .156 | 78.82 | 50.71 | 13.4 | 38.1 |
| General average..... | ..... | 1,895 | 11.313 | [b]3.638 | 1.521 | 7.751 | .979 | 1.532 | .289 | .161 | 69.29 | 42.44 | 20.5 | 42.7 |

[a] Including 1.38 grams of organic phosphorus added to the food per period.
[b] Including 1.42 grams of inorganic phosphorus added to the food per period.

The amount of nitrogen ingested per 1,000 grams of body weight for a seven-day period was as follows: No. 1, 6.7 grams; No. 3, 6.8 grams; No. 4, 5.2 grams. The amount of nitrogen absorbed per 1,000 grams of body weight per period of seven days was likewise very uniform, excepting for rabbit No. 2, being 5.6 grams for No. 1 and No. 3, 4.8 grams for No. 4, and 3.2 grams for No. 2. The amounts of nitrogen excreted in the urine and feces show considerable variation, the ratio of urine nitrogen to feces nitrogen being highest in No. 4, that is, 8.7:1, and lowest in No. 2, 2.6:1. No. 1 showed a ratio of 3.9:1, and No. 3 a ratio of 3.4:1.

In Table IV the ratios of nitrogen to phosphoric acid, calcium to magnesium, and phosphoric acid to calcium in food, feces, and urine are given. The relation of urine nitrogen to urine phosphorus is highest in the cases of the rabbits fed organic phosphorus. This is due to a larger excretion of phosphorus in the urine of rabbits Nos. 3 and 4, fed on inorganic phosphorus. This ratio in the feces is very regular, being 1.5:1 in three cases and for No. 2 increasing to 2.1:1. A higher ratio of calcium to magnesium is noted in the feces of the rabbits fed inorganic phosphorus. This ratio varies considerably in the urine of the individual rabbits.

TABLE IV.—*Ratios of calcium, magnesium, and phosphorus in food, feces, and urine—Principal period.*

RABBITS FED ORGANIC PHOSPHORUS.

| Rabbit No. | In— | $N:P_2O_5$. | Ca:Mg. | $P_2O_5$:Ca. |
|---|---|---|---|---|
| 1 | Food | 3.2 | 2.6 | 3.6 |
| | Feces | 1.5 | 2.7 | 2.0 |
| | Urine | 7.7 | 3.8 | 10.9 |
| 2 | Food | 3.2 | 3.0 | 3.2 |
| | Feces | 2.1 | 1.9 | 3.1 |
| | Urine | 5.7 | 1.0 | 14.7 |

RABBITS FED INORGANIC PHOSPHORUS.

| | | | | |
|---|---|---|---|---|
| 3 | Food | 3.2 | 2.9 | 4.2 |
| | Feces | 1.5 | 3.5 | 3.6 |
| | Urine | 5.5 | 2.2 | 34.0 |
| 4 | Food | 2.9 | 2.9 | 4.0 |
| | Feces | 1.5 | 3.7 | 1.8 |
| | Urine | 4.6 | 6.6 | 15.7 |

The figures show also that the phosphorus-calcium ratios of the food of rabbits Nos. 1 and 2 are lower than in the food of rabbits Nos. 3 and 4; this is due to a larger ingestion of phosphorus in the latter cases. The ratio of phosphoric acid to calcium in the feces varies with the individual case. The phosphoric acid to calcium ratios in the urine again show more phosphorus eliminated by rabbits Nos. 3 and 4 than by rabbits Nos. 1 and 2. The nitrogen and phosphoric acid eliminated in the urine are generally considered to represent the

amounts of the two substances metabolized by the system, but this does not hold in all cases. Rabbit No. 2, for example, which died, shows a much larger amount of katabolized nitrogen and phosphoric acid, as indicated by an increased elimination through the kidneys, but this does not indicate an increased metabolism of these two substances. The ratio of nitrogen to phosphoric acid excreted in the feces shows equal average figures for all the rabbits, excluding the figures for No. 2. If the figures for rabbit No. 2 are included, the average ratio is lower for the rabbits fed inorganic phosphorus. The bulk of the nitrogen (60 to 78 per cent) is eliminated by the kidneys, whereas, on the other hand, only 27 to 50 per cent of the phosphoric acid is thus eliminated. The percentage of absorbed nitrogen which was retained in the system is the same for rabbit No. 1 as for the average of Nos. 3 and 4, receiving inorganic phosphorus.

## PHOSPHORUS BALANCES.

The study of phosphoric acid metabolism raises many questions, of which the following are especially important:

(1) How is the phosphoric acid, ingested in different forms, taken up by the body?

(2) How is the phosphoric acid changed in the body?

(3) In what manner is the phosphoric acid eliminated from the body?

Many investigators have attempted to answer some or all of these questions, but no definite answer has been obtained. The generally accepted idea is that the phosphoric acid ingested in different forms is taken up by the body partly in various forms of organic combination and partly, also, in the inorganic or phosphate form. Most of the organic phosphorus taken up by the body—that absorbed from the intestines—is changed to the inorganic or phosphate form, and all such phosphorus is eliminated in the urine as phosphates. This idea that organic phosphorus compounds are more valuable than inorganic combinations of phosphoric acid has been promulgated in the medical literature during the past few years. Nevertheless, many practicing physicians continue to prescribe the inorganic forms, not only of phosphorus, but of iron, calcium, magnesium, etc. Yellow phosphorus is given solely as an alterative.

As the extent of the elimination of phosphoric acid is largely dependent upon the character of the food and the absorption of the phosphates in the intestines, it is apparent that the relationship between the nitrogen and phosphoric acid in the urine can only be approximately constant with a certain uniform food. Thus, on feeding dogs with an exclusive meat diet, as observed by Voit,[a] when the

[a] Cited by Hammarsten, A Textbook of Physiological Chemistry, rev. ed., New York, 1908.

nitrogen and phosphoric acid of the food exactly reappeared in the urine and the feces, the relationship was 8.1:1. In these experiments with rabbits the nitrogen and phosphoric acid ratio in the urine varied from 4.6:1 to 7.7:1. In starvation Wellmann[a] has shown that this relationship is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues, also another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this is the bone tissue. Tigerstedt[b] claims that only 0.134 gram of phosphoric acid is eliminated in the feces of man daily. For some years it was claimed by many investigators that the elimination of phosphorus and nitrogen should run parallel, both substances being derived from protein, the usual ratio of nitrogen to phosphorus being 7.5:1. In these experiments there is a general tendency in the individual data toward parallelism between the nitrogen and phosphoric acid excretion in the urine, but this ratio is not maintained in the general average, as in the case of the rabbits fed inorganic phosphorus a much larger proportion of the phosphoric acid is absorbed and eliminated by the kidneys. Siven,[c] Ehrström,[d] and Meyer[e] have also shown no parallelism to exist. Phosphorus is used in the formation of the bones and other bodies where no nitrogen is present. Moreover, the ratio of nitrogen and phosphoric acid will sometimes run as low as 3:1.

The amount of phosphoric acid which was fed to all the rabbits was considerably higher than the amount present in their normal diet. In fact, the food itself contained practically a sufficient amount to supply the needs of the system. The result is that by adding an excess of phosphoric acid metabolic changes were induced in all cases to a greater or less extent. The amount of phosphoric acid fed per seven-day period per 1,000 grams of body weight varied from 1.6 grams in rabbit No. 2 to 2.1 grams in the cases of rabbits Nos. 1 and 3. The amount of absorbed phosphorus per 1,000 grams of body weight was practically equal, 1.3 grams, excepting in the case of rabbit No. 2, where the figures show 0.7 gram of phosphoric acid per 1,000 grams of body weight. The ratio of phosphoric acid in the urine to that in the feces shows that the individual element was the most important factor, rabbit No. 4 eliminating a far larger proportional amount by the kidneys than in the case of any other rabbit. The percentage of phosphoric acid eliminated by the kidneys was higher in the rabbits fed inorganic phosphoric acid than in rabbit No. 1, but this simply means that more of the inorganic phosphoric acid passed through the kidneys unaltered, for rabbit No. 1 retained a larger proportion of its absorbed phosphorus than did either No. 3 or No. 4.

[a] Arch. gesam. Physiol., 1908, *121*:508.
[b] Skand. Arch. Physiol., 1904, *16*:67.
[c] Ibid., 1901, *11*:308.
[d] Ibid., 1903, *14*:82.
[e] Zts. physiol. Chem., 1904–5, *43*:1.

According to Ehrström,[a] who studied phosphorus elimination, from 50 to 88 per cent of the phosphoric acid is eliminated in the urine by the human organism, but the average amount given by different investigators varies from 70 to 80 per cent. With animal food almost all the phosphorus is eliminated in the urine, while with vegetable food a larger proportion of the phosphorus is found in the feces. The amounts of calcium and phosphorus present in the food stand in close relationship to one another. The rabbits eliminated from 27 to 50 per cent of the ingested phosphoric acid in the urine, a considerably lower percentage than in the case of carnivorous animals.

## ETHER-ALCOHOL-SOLUBLE PHOSPHORUS BALANCES.

The ether-alcohol soluble phosphorus balances were determined during the principal feeding period. The amount of phosphorus ingested in this form was practically the same in all cases. It is interesting to note the small portion of the 29 per cent of organic combined phosphorus in phytin, which is soluble in ether and alcohol. The figures show that 0.59 per cent of the phosphorus was present in phytin as ether-alcohol soluble phosphorus. This shows that the ether-alcohol extracted phosphorus may represent but a small proportion of the total organic combined phosphorus.

The presence of organic phosphorus in the urine has been discussed, but it is interesting to note that the average figures in Tables V and VI show a slightly larger amount of the so-called organic, or ether-alcohol soluble phosphorus in the urine in the case of the rabbits fed on inorganic phosphorus.

TABLE V.—*Ether-alcohol-soluble phosphoric acid balances—Principal period.*

RABBITS FED ORGANIC PHOSPHORUS.

| Date. | Number of rabbit. | Phosphoric acid ingested. | Excreted as phosphoric acid. | | | Phosphoric acid balance. | Total phosphoric acid in feces. | Ether-alcohol soluble phosphoric acid of feces in terms of total phosphoric acid. |
|---|---|---|---|---|---|---|---|---|
| | | | In urine. | In feces. | Total. | | | |
| *1905.* | | *Gram.* | *Gram.* | *Gram.* | *Gram.* | *Gram.* | *Grams.* | *Per cent.* |
| February 17–23 | 1 | 0.2800 | 0.0180 | 0.0146 | 0.0596 | 0.2380 | 0.6020 | 2.4 |
| February 24–March 1 | 1 | .2923 | .0050 | .0795 | .0845 | .2075 | 1.5680 | 5.1 |
| March 2–8 | 1 | .3081 | .0036 | .0992 | .1028 | .2053 | .9976 | 9.9 |
| March 9–15 | 1 | .3080 | .0000 | .1188 | .1188 | .1893 | 1.4008 | 8.1 |
| Average | | .2971 | .0067 | .0848 | .0914 | .2100 | 1.1571 | 6.4 |
| February 17–23 | 2 | .2413 | .0102 | .0873 | .1035 | .1378 | .9000 | 9.3 |
| February 24–March 1 | 2 | .2374 | .0110 | .1925 | .2035 | .0339 | 1.7380 | 20.8 |
| Average | | .2394 | .0136 | .1399 | .1535 | .0859 | 1.3190 | 15.1 |
| General average | | .2694 | .0102 | .1089 | .1225 | .1479 | 1.2381 | 10.8 |

[a] Skan. Arch. Physiol. 1903, *14*: 82.

TABLE V.—*Ether-alcohol-soluble phosphoric acid balances—Principal period*—Continued.

RABBITS FED INORGANIC PHOSPHORUS.

| Date. | Number of rabbit. | Phosphoric acid ingested. | Excreted as phosphoric acid. | | | Phosphoric acid balance. | Total phosphoric acid in feces. | Ether-alcohol soluble phosphoric acid of feces in terms of total phosphoric acid. |
|---|---|---|---|---|---|---|---|---|
| | | | In urine. | In feces. | Total. | | | |
| *1908.* | | *Gram.* | *Gram.* | *Gram.* | *Gram.* | *Gram.* | *Grams.* | *Per cent.* |
| February 17–23 | 3 | 0.2797 | 0.0133 | 0.0175 | 0.0308 | 0.2489 | 1.7664 | 1.0 |
| February 24–March 1 | 3 | .2797 | .0138 | .0232 | .0370 | .2427 | 1.4792 | 1.6 |
| March 2–8 | 3 | .2797 | .0080 | .0248 | .0328 | .2469 | 1.5308 | 1.6 |
| March 9–15 | 3 | .2797 | .0000 | .0090 | .0090 | .2707 | .5340 | 1.7 |
| Average | | .2797 | .0088 | .0187 | .0274 | .2523 | 1.3276 | 1.5 |
| February 17–23 | 4 | .2629 | .0081 | .0386 | .0467 | .2162 | .5920 | 6.5 |
| February 24–March 1 | 4 | .2797 | .0336 | .0166 | .0502 | .2295 | .4400 | 3.8 |
| March 2–8 | 4 | .2797 | .0180 | .0930 | .1110 | .1687 | .7600 | 12.2 |
| March 9–15 | 4 | .2797 | .0000 | .0322 | .0322 | .2477 | .7280 | 4.4 |
| Average | | .2755 | .0149 | .0451 | .0600 | .2130 | .6300 | 6.7 |
| General average | | .2776 | .0119 | .0319 | .0437 | .2327 | .9788 | 4.1 |

The figures also show that in the case of the rabbits fed organic phosphorus a considerable amount of ether-alcohol soluble phosphorus is excreted in the feces. The analyses of the feces of the rabbits fed inorganic phosphorus show that there is some ether-alcohol soluble phosphorus always present. This amount must come from the ether-alcohol soluble phosphorus of the food, or from the secretions of the intestinal juices. There is no doubt that the feeding of phytin, which contains 0.59 per cent of phosphorus in this form, greatly increases the amount of ether-alcohol soluble phosphorus in the feces. The insoluble calcium phosphate formed in the gastro-intestinal tract of the rabbits fed inorganic phosphorus may tend to give a higher ratio of ether-alcohol soluble phosphorus in the feces of the rabbits fed with phytin. Further, the results indicate that of the total phosphorus eliminated in the feces the percentage of ether-alcohol soluble phosphorus is much larger in the case of the rabbits fed on organic phosphorus. This may indicate that when phytin is fed in large amounts the gastro-intestinal tract is not able to split and absorb it all. Consequently the percentage of ether-alcohol soluble phosphorus in the feces is increased. The question of the presence of organic phosphorus in the urine is still unsettled. In the principal feeding period the phosphorus in the urine was determined by the uranium acetate titration method, and, further, the lecithin phosphorus, ether-alcohol method, was applied to the urine after evaporating 100 cc to dryness. The figures which are given in Table VI show that the urine results for phosphorus obtained

by the different methods are practically the same whether organic or inorganic phosphorus is fed.

TABLE VI.—*Various forms of phosphorus in urine—Principal period.*

| Date. | Number of rabbit. | Phosphoric acid per 100 cc of urine. | | | | Difference between two figures for organic phosphorus. |
|---|---|---|---|---|---|---|
| | | Total (by Neumann method). | Inorganic (by uranium acetate method). | Organic (by difference). | Organic (by ether-alcohol extraction). | |
| *1908.* | | *Gram.* | *Gram.* | *Gram.* | *Gram.* | *Gram.* |
| February 17–23 | 1 | 0.0986 | 0.1009 | −0.0023 | 0.0020 | 0.0043 |
| February 24–March 1 | 1 | ..... | .1153 | ..... | .0305 | ..... |
| March 2–8 | 1 | ..... | .1080 | ..... | .0303 | ..... |
| March 9–15 | 1 | ..... | .0670 | ..... | .0000 | ..... |
| February 17–23 | 2 | .1376 | .1308 | .0068 | .0017 | .0051 |
| February 24–March 1 | 2 | .1230 | .1184 | .0046 | .0011 | .0035 |
| February 17–23 | 3 | .1416 | .1277 | .0139 | .0014 | .0125 |
| February 24–March 1 | 3 | .1300 | .1287 | .0013 | .0012 | .0001 |
| March 2–8 | 3 | .1325 | .1290 | .0035 | .0008 | .0027 |
| February 17–23 | 4 | .2250 | .2039 | .0211 | .0009 | .0202 |
| February 24–March 1 | 4 | .1835 | .1740 | .0095 | .0032 | .0063 |
| March 2–8 | 4 | .1540 | .1340 | .0200 | .0018 | .0182 |
| March 9–15 | 4 | .1570 | .1290 | .0280 | ..... | ..... |

An ether-alcohol extraction of an inorganic phosphate solution (100 cc of a microcosmic salt solution) was made containing 0.2 gram of phosphoric acid and this gave 0.0054 gram of phosphoric acid by the ether-alcohol extraction method, as large an amount of ether-alcohol soluble phosphorus as was obtained in the average samples of the urine examined. This fact points to the conclusion that no ether-alcohol soluble phosphorus is normally present in the urine of rabbits, even after the feeding of organic phosphorus for several months.

A review of this question of the presence of organic phosphorus in the urine in general supports this conclusion. Ronald [a] was the first to call attention to the presence of organic phosphorus in the urine, and Rockwood [b] claims to have found phosphocarnic acid present. Bergmann,[c] however, using glycero-phosphoric acid made subcutaneous injections on sheep, but could not detect the same in the urine. Patten, Jordan, and Hart [d] in their extensive experiments with cows found no organic phosphorus eliminated in the urine, and like results were obtained by Mendel and Underhill [e] and by Le Clerc and Cook [f] working with rabbits and a dog.

[a] Philosophical Transactions, 1864, p. 461.
[b] Abs., Chem. Centrbl., 1895 (1), p. 1063.
[c] Arch. exper. Path. Pharm., 1902, *47*:77.
[d] Amer. J. Physiol., 1906, *16*:268.
[e] Ibid., 1906, *17*:75.
[f] J. Biol. Chem., 1906, *2*:203.

Mandel and Oertel [a] made some experiments on man along this line, feeding first food poor in phosphorus and later food rich in phosphorus, but found no effect upon the amount of organic phosphorus in the urine. They conclude that the organic phosphorus of the urine is of endogenous origin. The inorganic phosphorus was determined by uranium acetate titration, the solution was boiled with hydrochloric acid and retitrated, the difference being the organic phosphorus.

Organic phosphorus was found in the human urine by Sotnitschewsky.[b] Oertel,[c] using the method of precipitating the inorganic phosphorus with calcium chlorid in ammoniacal solution, found organic phosphorus in the urine of seven men.

Keller [d] undertook some extensive experiments along this line in the case of infants, and he concluded that the food did not influence the amount of organic phosphorus in the urine. He starved himself and found the amount of organic phosphorus eliminated the first three days was constant, while there was an increased elimination the fourth day. This indicated that some highly organic phosphorized tissue—that is, the lymphocytes—were broken down.

Symmers [e] studied this question in various pathological cases—diseases of the nervous system, enteric fever, tuberculosis, diabetes, and lymphatic leucaemia—and found large amounts of organic phosphorus in the urine.

In agreement with most investigators who have studied phytin and its action on the body, Scofone [f] and Giacosa [g] claim that phytin is principally eliminated in inorganic combinations.

There is no doubt that in pathological cases there is considerable organic combined phosphorus eliminated, but there is much doubt as to whether any phosphorus in the organic form is eliminated normally in the urine. The methods which have been employed to separate the two forms of phosphorus are far from satisfactory. The slight difference between two results obtained by different methods, which in many cases would be counted as duplicates, has been classed as due to the presence of organic phosphorus. Moreover, in this work, ether-alcohol soluble phosphorus was found in the urine. This is of little significance, because a solution of sodium hydrogen phosphate yielded an equivalent or greater amount of ether-alcohol soluble phosphorus

[a] N. Y. Univ. Bul. Med. Sci., 1901, *1* : 165.
[b] Zts. physiol. Chem., 1880, *4* : 214.
[c] Ibid., 1898–9, *26* : 123.
[d] Ibid., 1900, *29* : 146.
[e] J. Path. Bact., 1904–5, *10* : 159, 427.
[f] Abs., Biochem. Centrbl., 1905, *3* : 606.
[g] Ibid., 1905, *4* : 572.

by this same method, which precludes the possibility of any phosphorus in this form being normally present in the urine of rabbits.

In Table VI, where the total phosphorus is determined by the Neumann method and the inorganic phosphorus by titration with uranium acetate, the difference is called organic phosphorus. That the uranium acetate method is not absolutely correct is indicated by the figures which in several cases show more phosphorus by this method than by the Neumann method. In the case of rabbit No. 4 and in one instance in the case of rabbit No. 3 the differences are too large to be explained on the basis of experimental error. It is certain from these results that the ingestion of organic phosphorus does not cause an increased elimination of organic phosphorus in the urine; but the fact that in the case of the rabbits fed inorganic phosphorus there should be an apparent elimination of organic phosphorus in the urine in some cases must be explained on the basis of the endogenous origin of the organic phosphorus of the urine which appears to take place only in abnormal cases.

### CALCIUM AND MAGNESIUM BALANCES.

During the principal metabolism experiments, lasting four weeks, the calcium and magnesium balances were determined in addition to those of nitrogen and phosphoric acid. The amount of calcium ingested per seven-day period varied from 0.44 to 0.58 gram per 1,000 grams body weight, while that of magnesium varied from 0.15 to 0.21 gram, rabbit No. 1 receiving more than rabbits Nos. 3 and 4, while rabbit No. 2, which died at the end of the first two weeks, received about the same amount as rabbits No. 3 and No. 4. Goitein [a] states that unless a rabbit receives 0.16 gram of calcium per kilo body weight, a loss of calcium will occur. The figures show that the rabbits under this observation received far more than that minimum amount and, therefore, were in no danger of calcium starvation.

The figures in Table VII show that the calcium excreted in the urine was 9 per cent for No. 1, 10.8 per cent for No. 2, 4.2 per cent for No. 3, and 4.9 per cent for No. 4. In the case of the rabbits fed organic phosphorus, the average amount of calcium absorbed from the intestinal tract or metabolized was higher than in the case of those fed inorganic phosphorus. These figures agree with the theory that the calcium and phosphorus in the inorganic form unite to form the insoluble calcium phosphate, which is eliminated by the bowels in unchanged form.

[a] Arch. gesam. Physiol., 1906, *115* : 118.

TABLE VII.—*Calcium and magnesium balances—Principal period.*

RABBITS FED ORGANIC PHOSPHORUS.

| Date. | Rabbit No. | Total ingested. | | Excreted in urine. | | Excreted in feces. | | Total excreted. | | Daily balance. | | Daily balance ratio. | Excreted in urine. | | Absorbed material retained. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ca. | Mg. | Ca. | Mg. | Ca. | Mg. | Ca. | Mg. | Ca. | Mg. | Ca.: Mg. | Ca. | Mg. | Ca. | Mg. |
| *1908.* | | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | | *P. ct.* | *P. ct.* | *P. ct.* | *P. ct.* |
| February 17–23. | 1 | 0.888 | 0.330 | 0.029 | 0.006 | 0.407 | 0.139 | 0.436 | 0.145 | 0.065 | 0.026 | 1:0.40 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 1 | .863 | .320 | .166 | .053 | .771 | .257 | .937 | .310 | .011 | .002 | 1:0.18 | ..... | ..... | ..... | ..... |
| March 2–8..... | 1 | .910 | .352 | .057 | .011 | .528 | .209 | .585 | .220 | .047 | .019 | 1:0.40 | ..... | ..... | ..... | ..... |
| March 9–15..... | 1 | .910 | .352 | .068 | .013 | .551 | .244 | .619 | .257 | .049 | .014 | 1:0.29 | ..... | ..... | ..... | ..... |
| Average..... | 1 | .893 | .339 | .080 | .021 | .564 | .212 | .644 | .233 | .037 | .015 | 1:0.32 | 9.0 | 6.2 | 75.6 | 81.8 |
| February 17–23. | 2 | .858 | .279 | .118 | .119 | .363 | .158 | .481 | .277 | .053 | .000 | ..... | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 2 | .735 | .250 | .054 | .043 | .488 | .283 | .542 | .326 | .031 | −.011 | ..... | ..... | ..... | ..... | ..... |
| Average ..... | 2 | .796 | .264 | .086 | .081 | .425 | .221 | .512 | .301 | .042 | −.006 | ..... | ..... | ..... | ..... | ..... |
| General av'ge ... | ... | .844 | .301 | .083 | .051 | .494 | .216 | .578 | .267 | .040 | .005 | ..... | ..... | ..... | ..... | ..... |

RABBITS FED INORGANIC PHOSPHORUS.

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| February 17–23. | 3 | 0.893 | 0.307 | 0.046 | 0.006 | 0.669 | 0.223 | 0.715 | 0.229 | 0.025 | 0.011 | 1:0.44 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 3 | .893 | .307 | .029 | .046 | .281 | .100 | .310 | .146 | .083 | .023 | 1:0.29 | ..... | ..... | ..... | ..... |
| March 2–8..... | 3 | .893 | .307 | .027 | .009 | .390 | .040 | .417 | .049 | .068 | .037 | 1:0.54 | ..... | ..... | ..... | ..... |
| March 9–15..... | 3 | .893 | .307 | .049 | .008 | .133 | .056 | .182 | .064 | .102 | .035 | 1:0.34 | ..... | ..... | ..... | ..... |
| Average..... | ... | .893 | .307 | .038 | .017 | .368 | .105 | .368 | .122 | .069 | .027 | 1:0.40 | 4.2 | 5.5 | 94.6 | 91.5 |
| February 17–23. | 4 | .788 | .258 | .020 | .005 | .285 | .098 | .305 | .103 | .069 | .022 | 1:0.32 | ..... | ..... | ..... | ..... |
| February 24–March 1..... | 4 | .893 | .307 | .050 | .015 | .261 | .063 | .311 | .078 | .083 | .033 | 1:0.40 | ..... | ..... | ..... | ..... |
| March 2–8..... | 4 | .893 | .307 | .060 | .021 | .419 | .106 | .479 | .127 | .059 | .026 | 1:0.44 | ..... | ..... | ..... | ..... |
| March 9–15..... | 4 | .893 | .307 | .043 | .025 | .394 | .096 | .431 | .121 | .065 | .026 | 1:0.40 | ..... | ..... | ..... | ..... |
| Average..... | ... | .867 | .296 | .043 | .017 | .339 | .091 | .382 | .107 | .069 | .027 | 1:0.39 | 4.9 | 5.7 | 91.9 | 91.7 |
| General av'ge ... | ... | .880 | .302 | .041 | .017 | .354 | .095 | .376 | .115 | .069 | .027 | 1:0.40 | 4.6 | 5.6 | 93.3 | 91.6 |

In the case of man, from 5 to 10 per cent of the calcium is normally excreted in the urine; the remainder is excreted with the feces either directly, or a part may be absorbed from the small intestine and excreted into the large intestine, as shown by Voit[a] in the case of the dog. Some calcium may come from the intestinal juices. According to Schetelig,[b] Von Noorden,[c] and Rumpf,[d] the amount of calcium excreted in the urine increases with the amount of water taken into the system. Various authors have increased the calcium elimination by adding acids (especially hydrochloric acid) and salts to the food. Hoppe-Seyler[e] and Von Noorden found an increased calcium elimination during complete rest. A calcium retention was found by Rumpf, Hirschler, and Terray[f] in feeding milk (containing 1.58 grams of calcium per liter). The total volume of urine in each case

[a] Cited, Hammarsten, Textbook of Physiological Chemistry, rev. ed., New York, 1908.

[b] Virchow's Archiv, 1880, *82*:437.

[c] Beitr. Lehre Stoffwechsel gesund. krank. Menschen, Berlin, 1902.

[d] Berlin. klin. Wochenschr., 1897, *34*:265.

[e] Zts. physiol. Chem., 1891, *15*:161.

[f] Zts. klin. Med., 1905, *57*:137.

was practically the same, varying only from 975 to 1,100 cc per period on an average, and yet the calcium excreted was subject to considerable variation even in the case of the same rabbit fed on the same diet for several weeks. This fact is contrary to the findings of Patterson,[a] who found that on a fixed diet with man the urinary calcium ran parallel to the total amount of urine excreted. Although there was a smaller amount of calcium metabolized by the rabbits fed inorganic phosphorus, yet of this amount a larger proportion was retained than in the case of those fed organic phosphorus.

By excluding the very abnormally high amount of magnesium found in the urine in the case of rabbit No. 2 (which died), we find a very close agreement in the case of the other three animals, though there is a slight tendency for the rabbit fed organic phosphorus to excrete more magnesium in the urine. The amount of metabolized magnesium that was retained shows that the rabbits fed inorganic phosphorus, while metabolizing a smaller amount of the magnesium than did those fed organic phosphorus, retained a larger per cent of the amount actually metabolized. In man from 29 to 38 per cent of the ingested magnesium is excreted in the urine, which is higher than in the case of rabbits. The ratio of calcium to magnesium eliminated in the urine is not constant in the cases studied. According to Bertram[b] and Renvall,[c] 29 to 38 per cent of magnesium is excreted in the urine and 62 to 71 per cent is eliminated in the feces. It is more easily excreted through the kidneys than is calcium. The ratio of calcium oxid to magnesium oxid excreted in human urine, according to Klemperer and Tritschler,[d] varies from 1:0.8 to 1:1.2. The ratio of calcium to magnesium excreted in the feces in the cases of rabbits Nos. 1 and 2 is lower than in the case of Nos. 3 and 4. The ratio of calcium to magnesium excreted by man is held to be 8:1, but in the case of the rabbits the ratio is considerably lower. The amount of magnesium required by man is placed at 0.6 gram per day. In all cases of the rabbits experimented with, daily positive calcium and magnesium balances were obtained.

## CHEMICAL ANALYSIS OF THE BODIES OF THE RABBITS.

In all cases the analyses were made on composite samples of two rabbits, and represent the average figures. All analyses were calculated to a water-free basis.

### BONES.

The bones were freed from the adhering muscular and tendon tissue and placed in a large kettle and boiled for several hours with water until all the flesh could be easily removed. They were then dried in a hot-air bath, again scraped, and finally ground into a fine powder. The bone powder in the case of the two normal rabbits was

[a] Bio-Chem. J., 1908, *3*:39.
[b] Abs. Chem. Centrbl., 1897, *68*:957.
[c] Skand. Arch. Physiol., 1904, *16*:94
[d] Zts. klin. Med., 1902, *44*:337.

lighter in color and did not have the oily feeling characteristic of that of the phosphorus-fed rabbits. The water content of the normal bones was lower than in the cases where phosphorus was fed. This was due to the higher fat content in those cases. The remainder of the figures represent the results calculated to a water-free basis, and are shown in Table VIII.

TABLE VIII.—*Chemical analyses of bodies of rabbits (water-free basis).*

| Description of sample. | Nitrogen. | Ash. | Calcium. | Magnesium. | Ether extract. | Phosphoric acid. | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Total. | Ether-alcohol soluble. | Ether-alcohol soluble in terms of total. |
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| **RABBITS FED ORGANIC PHOSPHORUS, NOS. 1 AND 2.** | | | | | | | | |
| Bones | 4.53 | 55.56 | 8.86 | 0.22 | 11.33 | 23.96 | 0.055 | 0.23 |
| Livers | 7.64 | 3.78 | .00 | .00 | 44.95 | 1.98 | .680 | 34.34 |
| Blood | 14.72 | 5.64 | Trace. | .00 | Trace. | .75 | .008 | 1.07 |
| Brains | 6.12 | 7.00 | .31 | .09 | 43.23 | 3.96 | 2.350 | 59.34 |
| Nerves | 5.52 | 6.54 | .21 | .11 | 46.96 | 3.72 | 2.390 | 64.26 |
| Teeth | ..... | 74.65 | 24.73 | 1.35 | ..... | 35.31 | ..... | ..... |
| **RABBITS FED INORGANIC PHOSPHORUS, NOS. 3 AND 4.** | | | | | | | | |
| Bones | 4.68 | 55.92 | 7.74 | 0.15 | 9.39 | 26.33 | 0.061 | 0.23 |
| Livers | 9.41 | 4.73 | .00 | .00 | 34.48 | 2.56 | .854 | 33.36 |
| Blood | 14.42 | 4.72 | Trace. | .00 | Trace. | .69 | .008 | 1.16 |
| Brains | 6.52 | 7.17 | .27 | .06 | 43.89 | 4.07 | 1.160 | 28.51 |
| Nerves | 3.72 | 5.83 | .28 | .06 | 47.34 | 4.28 | 1.470 | 34.35 |
| Teeth | ..... | 76.10 | 24.65 | 1.22 | ..... | 34.70 | ..... | ..... |
| **NORMALLY FED RABBITS, NOS. 5 AND 6.** | | | | | | | | |
| Bones | 4.32 | 57.47 | 10.17 | 0.23 | 5.12 | 25.93 | 0.069 | 0.27 |
| Livers | 11.90 | 5.67 | .00 | .00 | 14.47 | 2.85 | 1.090 | 38.24 |
| Blood | 14.42 | 4.55 | .44 | .19 | Trace. | .58 | .037 | 6.33 |
| Brains | 6.85 | 7.82 | .51 | .08 | 38.50 | 4.16 | 1.750 | 42.07 |
| Nerves | 4.39 | 5.97 | .44 | .12 | 44.34 | 3.90 | 2.310 | 59.23 |
| Teeth | ..... | 75.17 | 28.35 | 1.15 | ..... | 35.63 | ..... | ..... |

The normal rabbits show a slightly higher figure for total ash in the bones than do the phosphorus-fed rabbits. Several investigators have pointed out the fact that the skeleton becomes poorer in water and richer in ash with age. Voit[a] showed this fact in the case of dogs, and Brubacher[b] in the case of children. There is a considerable variation in the ratio of the calcium to the total ash, even in normal animals. Wellmann[c] states that in cases of starved rabbits the bones show a smaller percentage of organic matter, that is, a higher ratio of ash. In the case of the normal rabbits, where the highest ash is present, we expect to find the highest percentage of ash constituents, and such is the case.

[a] Cited, Hammarsten, Textbook of Physiological Chemistry, rev. ed., New York, 1908.
[b] Zts. Biol., 1890, *27* : 517.
[c] Arch. gesam. Physiol., 1908, *121* : 508.

The amounts of ether-soluble matter present in the bones show some very interesting results. Both when organic and inorganic phosphorus were fed, the bones contained more ether-soluble matter than the normal bones, evidently a case similar to the increased percentage of ether-soluble material in the liver and presumably due to the phosphorus fed. The bones of the normal rabbits and of those fed inorganic phosphorus show practically an equal amount of total phosphorus, the figure for the rabbits fed organic phosphorus being a little lower. The bones of the normally fed rabbits contain a slightly higher percentage of ether-alcohol soluble phosphorus than in the other cases. The conclusion is that feeding this large amount of phosphorus has not materially affected the quantity of phosphorus stored in the bones, nor has the form of that storage been appreciably changed. There is 0.27 per cent of the total phosphorus as ether-alcohol soluble phosphorus in the bones of the normal rabbits, and 0.23 per cent in the other cases, but the difference, 0.04 per cent, is not large enough to have any significance. In a matter of this kind the age of the animal should be taken into account, for it is known that there is less calcium phosphate and more carbonate in the bones of old rabbits than in the bones of young ones; likewise, less calcium, magnesium, and phosphorus in the bones of starved rabbits, as shown by Wellmann.

On a fat-free, water-free basis the analysis of the bones, given in Table IX, shows a slight variation in the ash content, the ash of the rabbits fed organic phosphorus being slightly higher than that for the bones of the rabbits fed inorganic phosphorus, which in turn is higher than the normal. In regard to the calcium and magnesium, the bones of the rabbits fed inorganic phosphorus contain the smallest amount, while the highest percentage of calcium is present in the normal bones. The total ash content in the cases of the experimental rabbits was greater than in the normal rabbits. The bones of the rabbits fed inorganic phosphorus show a higher phosphorus content than in the other cases; but the results as regards ether-alcohol soluble phosphorus show little variation, the bones of the normal rabbits containing the largest proportion and those of the rabbits fed organic phosphorus the smallest proportion.

TABLE IX.—*Analysis of bones calculated to a fat- and water-free basis.*

| Rabbits. | Calcium. | Magnesium. | Ash. | Phosphoric acid. | | | |
|---|---|---|---|---|---|---|---|
| | | | | Total. | Ether-alcohol soluble. | Ether-alcohol insoluble. | Ether-alcohol soluble in terms of total. |
| | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| Fed organic phosphorus | 9.99 | 0.25 | 62.06 | 27.04 | 0.065 | 26.97 | 0.26 |
| Fed inorganic phosphorus | 8.54 | .17 | 61.71 | 29.06 | .067 | 28.99 | .23 |
| Normal | 10.72 | .24 | 60.57 | 27.33 | .073 | 27.26 | .28 |

LIVERS.

The livers of the rabbits were dried and powdered. The total ash of the livers of the normal rabbits is higher than the ash of the livers of those fed inorganic phosphorus which, in turn, is slightly higher than the ash of the livers of those fed organic phosphorus.

The changes produced in the livers do not seem to include any large storage of phosphorus in the organ, for the livers of the rabbits fed organic phosphorus which show the largest excess of fat contain the lowest percentage of total and ether-alcohol-soluble phosphorus, and the livers of the rabbits fed inorganic phosphorus, which have a lower fat content, contain a lower percentage of phosphorus than the normal rabbits' livers. Not only is there a difference in the total phosphorus content of the various livers, but the livers of the rabbits fed organic phosphorus contain a lower percentage of ether-alcohol-soluble phosphorus than is present in the other cases, the normal livers showing the highest figures. That there is an excess of fat in the livers of the rabbits fed an excessive amount of phosphorus, whether organic or inorganic, is shown by the figures for the ether-soluble matter, namely, 44.95 per cent in the case of the rabbits fed organic phosphorus, 34.48 per cent in the livers of those fed inorganic phosphorus, and only 14.47 per cent in the case of the livers of the normally fed rabbits.

TABLE X.—*Analysis of rabbits' livers (water-free basis).*

| No. of rabbit. | Weight of rabbit. | Ration. | Weight of liver. | | Percentage composition. | | | Actual amounts. | | Calculated to a fat-free basis. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Nitrogen. | | Phosphoric acid. | |
| | | | Moist. | Dry. | Nitrogen. | Phosphoric acid. | Ether extract. | Total nitrogen. | Total phosphoric acid. | Total. | Per 1,000 grams body weight. | Total. | Per 1,000 grams body weight. |
| | *Gms.* | | *Gms.* | *Gms.* | *P.ct.* | *P.ct.* | *P.ct.* | *Gms.* | *Gm.* | *Gms.* | *Gms.* | *Gms.* | *Gm.* |
| 1 | 1,550 | Organic phosphorus | 91.0 | 36.1 | 7.64 | 1.98 | 44.95 | 2.76 | 0.72 | 5.01 | 3.23 | 1.31 | 0.85 |
| 3 | 1,800 | Inorganic phosphorus | 81.5 | 27.5 | 9.41 | 2.56 | 34.48 | 2.58 | .70 | 3.94 | 2.18 | 1.07 | .60 |
| 4 | 2,000 | do | 86.0 | 35.0 | 9.41 | 2.56 | 34.48 | 3.29 | .90 | 5.02 | 2.50 | 1.37 | .69 |
| 5 | 2,050 | Normal | 59.0 | 14.6 | 11.90 | 2.85 | 14.47 | 1.74 | .42 | 2.04 | 1.00 | .49 | .24 |
| 6 | 2,200 | do | 60.0 | 15.0 | 11.90 | 2.85 | 14.47 | 1.79 | .43 | 2.09 | .95 | .50 | .23 |

In Table X the results for the body weights, liver weights, total nitrogen, total phosphorus, and ether extract found in the livers of the various rabbits are given. That phosphorus in the forms fed tends to enlarge the livers is seen from the percentage of total weight of the livers in terms of the body weight of the rabbits. In the case of Nos.

5 and 6 (the normal rabbits) the livers formed 3 and 2.8 per cent, respectively, of the total weight of the rabbits, while in Nos. 1, 3, and 4 the liver weight made 6.0, 4.5, and 4.4 per cent of the body weight. More total nitrogen and phosphorus is present in the livers of phosphorus-fed rabbits than in the normal livers, and calculated to a fat-free basis the differences are more evident. The tendency of organic phosphorus to produce fat is especially striking; in these experiments an increased amount of ether-soluble matter is seen in the bones, livers, brains, and nerves of the phosphorus-fed rabbits. Jordan, Patten, and Hart,[a] in their experiments with phytin, state that when the cows were reduced from a high to a low phytin diet, the percentage of fat in the milk was reduced. The fact that phytin tends to the production of fat in various organs and secretions seems to be established by all of these experiments.

### BLOOD.

The figures for blood analysis are given on a water-free basis. The amount of ash is highest in the blood of the rabbits fed organic phosphorus. The blood of those fed inorganic phosphorus and of the normal rabbits contain about an equal amount of ash. The variations are not large enough to be of any consequence when the individuality of the different rabbits is considered. The calcium content of the blood of all the phosphorus-fed rabbits is reduced, showing only a trace, while 0.44 per cent of calcium is present in the dried blood of the normal rabbits. The same holds true in the case of the magnesium, 0.19 per cent being present in the dried blood of the normal rabbits and none being found in the blood of the phosphorus-fed rabbits. The prevailing belief is that the composition of the blood changes but little if any, no matter what changes may take place in the body tissues. Patterson[b] found, in the case of animals kept on a calcium-poor diet, that the ratio of the calcium of the blood to the total ash of the blood was the same as that of normal animals. The amount of total phosphorus present in the normal blood is somewhat lower than in the cases where phosphorus was fed. The ether-alcohol soluble (lecithin) phosphorus is highest in the blood of the normal rabbits and the figures show that 6.33 per cent of the total phosphorus is present in the dried blood of the normal rabbits as ether-alcohol soluble phosphorus against 1.07 and 1.16 per cent in the case of the experimental rabbits. Here again we see that the constant ratio of the constituents of the blood is not maintained. Only traces of ether-soluble matter were found in any of the samples of blood examined. (Table VIII.)

[a] Amer. J. Physiol., 1906, *16* : 268.

[b] Bio-Chem. J., 1908, *3* : 39.

### BRAINS.

The brains of the rabbits were completely removed and dried in platinum dishes, then ground in a mortar and mixed as well as possible before analyzing. The ash varied from 7.0 to 7.82 per cent, being highest in the normal brains. As the age and other factors influence the ash content of all organs, this variation has no significance. The percentage of calcium shows some variation and is likewise highest in the ash of the brains of the normal rabbits.

The percentage of magnesium remains fairly constant in all cases. The ratio of calcium to magnesium in the brains is 6.3:1 in the normal rabbits, 4.5:1 in the brains of those fed inorganic phosphorus, and 3.5:1 in the brains of those fed organic phosphorus.

The total phosphorus found in the brains is about the same in all cases, but this is not true of the ether-alcohol soluble phosphorus. The brains of the rabbits fed organic phosphorus show 2.35 per cent phosphorus as ether-alcohol soluble, or 59.34 per cent of the total. The brains of those fed inorganic phosphorus show but 1.16 per cent phosphorus as ether-alcohol soluble phosphorus, or 28.51 per cent of the total. This is lower than the figures for brains of the normal rabbits, which contain 42.07 per cent of the phosphorus in a form soluble in ether and alcohol. It appears from these figures that the brains of the rabbits fed organic phosphorus contain an appreciably larger amount of ether-alcohol soluble phosphorus than do the brains of rabbits fed on inorganic phosphorus or those of the normally fed rabbits. (Table VIII.)

### NERVES.

The ash content on a water-free basis shows a fairly constant percentage in all cases. As in the brain, the calcium content of the nerves is higher for the normal rabbits than in the other cases.

The amount of magnesium in the nerves of the normal rabbits is 0.12 per cent, 0.11 per cent, or practically the same, in the nerves of those fed organic phosphorus, and one-half, or 0.06 per cent in the nerves of those fed inorganic phosphorus.

The ratio of the calcium to the magnesium is much lower in the nerves of the rabbits fed organic phosphorus than in the other cases. The data for ether-soluble material are fairly uniform, being a trifle lower for the nerves of the normal rabbits than for the experimental rabbits.

The total phosphorus content varies from 3.72 per cent in the case of the rabbits fed organic phosphorus to 4.28 per cent in the case of those fed inorganic phosphorus. The amount of ether-alcohol soluble phosphorus is lowest in the case of the rabbits fed on inorganic phosphorus, the percentage of the total phosphorus in this form being

34.25 per cent against 64.26 per cent in the case of the rabbits fed on organic phosphorus, and 59.23 per cent in the normal rabbits. These results are similar to those recorded in the case of the brains. (Table VIII.)

TEETH.

The teeth were freed from adhering bone and muscle tissue by scraping and were dried in a hot-air oven at 100° C. The ash was constant in all cases, averaging 75 per cent. There was somewhat more calcium and a trifle less magnesium found in the teeth of the normal rabbits than in the teeth of the phosphorus-fed rabbits. These results run parallel with those obtained for calcium in the case of the bones of the rabbits. The total phosphorus was practically no higher in the normal rabbits than in the other cases. (Table VIII.)

INTESTINES.

Phosphorus was estimated in portions of the small intestines of the various rabbits. The loops of intestine were well washed and dried between filter papers and then in an air bath at 45° C. for five hours. When sufficiently dry they were cut into small bits. Moisture and total phosphorus were determined on a weighed quantity of this substance and on another portion the following extractions were made:

One gram of substance was ground fine with pure sand in a porcelain mortar and transferred to a 300 cc Erlenmeyer flask. Thirty cubic centimeters of absolute ether were then added and the whole extracted on the water bath overnight, using a reflux condenser. The ether extract was then filtered through a hardened filter into an ordinary Jena flat-bottom flask. Particles of residue found on the filter paper were scraped back into the Erlenmeyer flask. To this ether extract residue 60 cc of absolute alcohol were added and boiled for three hours, using a reflux condenser. This alcohol extract was filtered hot into the Jena flask containing the ether extract and the residue washed twice with separate portions of 25 cc of hot alcohol and the washings were added to the combined extract. Phosphorus was determined in the combined ether-alcohol filtrate by the Neumann method.

The ether-alcohol extraction residue was next treated six times with 50 cc portions of cold water saturated with chloroform, allowing ten hours for each extraction, using the same hardened filter paper as before and scraping back the residue from the filter paper into the flask. This solution was evaporated to dryness and the phosphoric acid determined therein by the Neumann method. This is called the phosphorus insoluble in ether and alcohol, but soluble in water. Phosphoric acid was then obtained in the residue by difference and called the phosphorus insoluble in ether, alcohol, and water.

In all cases a large amount of the phosphorus of the fresh sample was soluble in water, fully 50 per cent being dissolved by this means during the preparation of the sample. The amount of phosphorus insoluble in alcohol, ether, and water was higher in the normal rabbits' intestines than in the other cases. The amount of ether-alcohol soluble phosphorus showed but little variation.

## SUMMARY.

There are many variations in the composition of the different portions of the rabbits, apparently due to the feeding of phosphorus in the two forms. The amount of ether-alcohol-soluble phosphorus stored in the brains and nerves is much lower in the case of the rabbits fed inorganic phosphorus than in the normal rabbits and those fed organic phosphorus. The organic and the inorganic phosphorus, fed in excess, caused an increased percentage of ether-soluble material in the bones, and an indication of the same is noted in the brains and in the nerves. No increased storage of calcium or magnesium was noted; on the contrary there is a slight decrease, as compared with the normal, in bones, nerves, and brain. The ether-alcohol soluble phosphorus of the blood was reduced in all cases where phosphorus was fed. The increase in the fat of the livers was marked in both cases of phosphorus feeding, but was greater for the rabbits fed organic phosphorus. If a large percentage of ether-alcohol soluble phosphorus in the brains and nerves is desirable, then the rabbits fed organic phosphorus were in a better condition, according to the data, than were those fed on inorganic phosphorus.

The nitrogen content was determined in all of the samples, and is given in Table VIII calculated to a water-free basis. The percentage of nitrogen found in the bones varied from 4.32 per cent in the bones of the normal rabbits to 4.68 per cent in the bones of the rabbits fed on inorganic phosphorus. The percentage of nitrogen found in the livers of the experimental rabbits was considerably lower than in the livers of the normal rabbits, this agreeing with the other data showing a generally poor condition of the livers of the former. The percentage of nitrogen found in the blood was fairly constant, as is usually the case, and the same is true of the brains. Just why we find a variation in the amount of nitrogen present in the nerves and spinal cord is difficult to explain. More ash and more ether-alcohol-soluble phosphorus are found in the samples of nerves of the rabbits fed organic phosphorus, which also show the highest percentage of nitrogen. The ratio of phosphorus to nitrogen in the liver of rabbits is 1:14.7, according to Wellmann.[a] His figures are higher than the results obtained in this experiment, the ratio of phosphorus to nitrogen being, for normal rabbits 1:9.7, for rabbits fed organic phosphorus 1:8.8, and for those fed inorganic phosphorus 1:8.6.

[a] Arch. gesam. Physiol., 1908, *121*:508.

## FINDINGS OF AUTOPSIES.

At the conclusion of the principal feeding period the rabbits were chloroformed, autopsies were made, and histological slides of several of the organs prepared. Two normal rabbits were similarly treated. The normal rabbits had been fed on corn, oats, and vegetables for some time previously and had been kept in cages. There seems to be no constant relationship between the total weights and the percentages of solids present in the various organs, blood, brains, and nerves of the six rabbits examined. The autopsies showed the following results: [a]

*Rabbit No. 1, fed organic phosphorus.*

Intestines: Normal, containing food. Some fat distributed along the intestines.
Lymphatics: Apparently normal.
Kidneys: Apparently normal.
Spleen: Apparently normal.
Liver: Very light in color, possibly fatty infiltration. Appearance similar to that of No. 2.
Stomach: Contained food. Normal.
Heart: Normal.
Lungs: Showed a condition of anemia along edges.
After drying: Bones seemed oily. Much fat in liver; fat left in bottom of dish after drying.

*Rabbit No. 2, fed organic phosphorus.*

Intestines: Normal, except colon distended with large amount of feces; no congestion.
Lymphatics: Apparently normal.
Kidneys: Apparently normal.
Spleen: Apparently normal.
Liver: Somewhat enlarged, pale, with yellow tinge; seemed pathological.
Stomach: Full of food; appeared to have extended area of old hemorrhage on lesser curvature.
Heart: Apparently normal; post-mortem blood clot.
Lungs: Left, apparently normal; right, partly congested.
Nervous system: Normal.
General appearance: Good.

*Rabbit No. 3, fed inorganic phosphorus.*

Intestines: Apparently normal.
Lymphatics: Apparently normal.
Kidneys: There seemed to be slight irritation and congestion.
Liver: Light colored; enlarged.
Spleen: Normal in size and color generally; better condition than Nos. 1 and 2; yellow color; numerous small areas of what may be fatty degeneration or infiltration.
Stomach: Old hemorrhage around greater curvature.
Heart: Apparently normal.
Lungs: General appearance good, but apex of right lung had a small area of congestion.
General appearance: Fairly fat and in good condition externally.

[a] H. L. Amoss, of the Animal Physiological Laboratory, assisted in making the autopsies and interpreting the histological slides which were prepared by E. A. Read, of the Microchemical Laboratory.

*Rabbit No. 4, fed inorganic phosphorus.*

Intestines: Apparently normal.
Kidneys: Right, slight yellow color in cortex, pitted, congested; left, pitted over external surface, slight congestion in medulla, very slight yellowish color in cortex.
Spleen: Apparently normal.
Liver: Light color, lighter spots seen. Gall bladder apparently normal.
Stomach: Capillaries of fundus darkened—apparently not post-mortem change.
Heart: Apparently normal.
Lungs: Left, apparently normal; right, lower half of lower lobe congested.

*Rabbits Nos. 5 and 6, normally fed.*

The autopsies showed that in the cases of the two normal rabbits, Nos. 5 and 6, the organs were apparently normal. The livers had the normal dark-red color in contrast with the pale livers of Nos. 1, 2, 3, and 4.

The autopsies showed that none of the experimental rabbits was in a normal condition. The livers were especially affected, being pale and abnormal, and indicating an excess of fat.

The relation of the liver to metabolism is a problem important to both the physiologist and the pathologist. For a long time it has been recognized that one of the functions of the liver is connected in some way with the destruction or removal of poison from the blood, especially such poisons as are produced in, or absorbed from, the alimentary canal. It was early recognized that the destruction of the liver cells leads to serious poisoning, and this was experimentally demonstrated by Eck, who excluded the liver from the circulation by making a direct communication between the portal vein and the inferior vena cava, an operation known as "Eck fistula."

The question of fatty degeneration has an important bearing from the physiological point of view, for fatty degeneration is another proof of the formation of fat from proteins. From the investigations of Bauer[a] on dogs and Leo[b] on frogs we must admit that at least in acute poisoning by phosphorus fatty degeneration takes place with the formation of fats from proteins. Still, investigators are not unanimous on this point, and Pflüger[c] especially has raised objections to these experiments.

The ideas of the fatty degeneration of protein in the old sense as used by these writers have been changed by the work of Rosenfeld,[d] etc., who believe in the theory of fat transportation.

In the case of a phosphorized dog, Mandel[e] has shown that lactic acid disappears from the blood and urine when phlorhizin glycosuria

[a] Zts. Biol., 1871, *7* : 63.
[b] Zts. physiol. Chem., 1885, *9* : 469.
[c] Arch. gesam. Physiol., 1891–92, *51*: 317.
[d] Ergeb. Physiol., Pt. I, Biochemie, 1903, *2* : 50.
[e] Amer. J. Physiol., Proc., 1905, *13* : 16.

is induced. Lusk[a] offers the following general hypothesis as his explanation of fatty changes in tissues:

> The lactic acid which occurs is derived from the sugar formed in proteid metabolism. In the above case the sugar is removed before its conversion into lactic acid. In phlorhizin diabetes, dextrose does not burn; in phosphorus poisoning lactic acid derived from dextrose does not burn. In both cases a sugar-hungry cell, or one where carbohydrate is not oxidized, is found, and under these circumstances fat is attracted to the cell, and in larger quantities than can be useful. Wherever sugar freely burns this fatty infiltration is impossible. A reduced local circulation in a portion of the heart may produce anemia of the part, an imperfect local combustion of lactic acid normally formed and a fatty infiltration of the locality.
>
> * * * * * * *
>
> It has been stated that the action of phosphorus is to induce autolysis (self-digestion) of the body's protoplasm (Jacoby,[b] Waldvogel[c]), since leucin, tyrosin, and other amino acids may be eliminated in considerable quantity in the urine. Oswald[d] thinks that phosphorus destroys or weakens the antiautolytic agents of the body. That autolytic enzymes do not gain free control over the cells through the direct influence of phosphorus is proved by the work of Ray, McDermott, and Lusk.[e] These authors found that phosphorus injections raised the proteid metabolism of fasting dogs to 250, 260, 283, 248, 183, and 164 per cent of that of the dog when normal. They contrasted this increased proteid metabolism with that obtained in phlorhizin glycosuria, which is represented by increases to 540, 450, 340, and 340 per cent. When, however, they gave phlorhizin and obtained the increased metabolism, and then injected phosphorus, this was not followed by any marked increase in proteid metabolism. Under these circumstances phlorhizin glycosuria is the predominating factor, removing the dextrose produced from proteid. As regards phosphorus poisoning, Araki[f] believes that lactic acid accumulation is due to lack of oxygenation of the tissues caused by a slow heart beat, but not due to anemia. He does not believe the oxygen deprivation to be very pronounced. The writer offers the explanation that phosphorus may affect the enzyme which breaks up the lactic acid derived from dextrose, and the accumulation of this acid may prevent the action of some of the denitrogenizing enzymes; and further, its noncombustion may necessitate an increase of proteid metabolism.
>
> This theory is strengthened by the discovery of Schryver[g] that the addition of lactic acid favors the accumulation of amino acids in autolysis of the liver.

In this connection we must recognize the fact that the presence of amino bodies—leucin, tyrosin, etc.—in the liver in cases of phosphorus poisoning is well established. Abderhalden and Bergell[h] detected glycocoll in the urine of a rabbit poisoned with phosphorus. Wohlgemut[i] found phenylanin and arginin in the urine after a case of phosphorus poisoning. An altered quantitative relationship between

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[a] The Elements of the Science of Nutrition, Philadelphia, 1906.
[b] Zts. physiol. Chem., 1900, *30* : 174.
[c] Arch. klin. Med., 1905, *82* : 437.
[d] Biochem. Centrbl., 1905, *3* : 365.
[e] Amer. J. Physiol., 1899, *3* : 139.
[f] Zts. physiol. Chem., 1893, *17* : 310.
[g] Bio-Chem. J., 1906, *1* : 123.
[h] Zts. physiol. Chem., 1903, *39* : 464.
[i] Ibid., 1905, *44* : 74.

arginin, lysin, and histidin was noted in the liver of a phosphorus-poisoned dog by Wakeman.[a] The amount of arginin was considerably lower than the amount present in the liver of a normal dog.

In the experiments here reported there is no doubt that, due to organic and inorganic phosphorus, an alteration in the livers, brains, and nerves has taken place, accompanied by the presence of abnormally high percentages of fat.

### HISTOLOGICAL EXAMINATION.

The following results were obtained from the histological examination:

#### Rabbits Nos. 1 and 2, fed organic phosphorus.

Liver, No. 1: Extensive areas of fatty degeneration and fatty infiltration. Sections stained with hemotoxylin and eosin, Fleming's solution, and Soudan III.

Liver, No. 2: Not quite as extensive areas of fatty degeneration and fatty infiltration as in the case of No. 1. Chronic inflammatory processes seen and general areas of hemorrhages.

Lungs, No. 1: Several areas of focal necrosis noticed surrounding vein in a state of passive congestion. Practically all vesicles collapsed and walls congested. General red cell extravasation.

Stomach, No. 2: Very slight congestion.

#### Rabbits Nos. 3 and 4, fed inorganic phosphorus.

Liver, No. 3: Infiltration in certain areas, but not as far gone as Nos. 1 and 2. Certain areas showed little change. Slight cloudy swelling. Slight chronic inflammation, not so marked as No. 2.

Liver, No. 4: Little difference compared with Nos. 1 and 2. Some subacute inflammatory processes seen, also some fatty degeneration. Soudan III stain shows more fatty degeneration than is seen with Fleming's.

Lungs, No. 3: Pneumonia in stage of gray hepatization.

Lungs, No. 4: Not normal, but in better general condition than others. Lumen of artery nearly obliterated by fibrous tissue. Bronchitis. Slight general congestion.

Heart, No. 3: Pericarditis. Extensive fatty degeneration of myocardium.

Kidneys, No. 3: Marked congestion. Slight parenchymatous degeneration.

Kidneys, No. 4: Parenchymatous degeneration. Congestion more pronounced than in case of No. 3.

Stomach, No. 3: Apparently normal.

Stomach, No. 4: Apparently normal.

#### Rabbits Nos. 5 and 6, normally fed.

Livers: Practically normal. No fat present; small areas in which few nuclei did not stain as deeply as the rest, probably due to sectioning.

Lungs: Congestion throughout. A few areas of hemorrhages with superimposed inflammatory processes.

Kidneys: Slight intertubular hemorrhage.

Hearts: Slight myocarditis.

Stomachs: Apparently normal.

The results of the histological examination confirm the opinions formed at the time of the autopsy and show most marked fatty

[a] Zts. physiol. Chem., 1905, *44* : 335.

degeneration of the livers of rabbits Nos. 1 and 2. The liver of rabbit No. 4 also shows fatty degeneration, but less marked, while rabbits Nos. 5 and 6 show normal livers. That the harmful effects noted are due to the excessive amounts of phosphorus fed either organic or inorganic, is proven from the cases of the normal rabbits which, although likewise kept in cages, showed apparently normal livers. In reviewing the phosphorus literature it was noticed that in several cases rabbits fed on organic phosphorus were reported to have died of pneumonia, as did rabbit No. 2 in the experiments here recorded.

Six photomicrographs[a] are appended, which show sections of the livers of rabbits Nos. 1, 2, and 4. The tissue in the case of rabbit No. 3 was exhausted before an osmic acid slide was prepared and it was therefore impossible to give a reproduction in this case. The photomicrographs in which the fat is shown stained black in position demonstrate that degeneration as well as fatty infiltration has taken place in the livers of both of the organic and in one of the inorganic-phosphorus-fed rabbits.

The author is indebted to Doctor Mohler, of the Bureau of Animal Industry, for his interpretation of the microscopic slides, in regard to which he makes the following statement:

The section in the case of rabbit No. 1 (Pl. I) shows a more marked and advanced lesion of fatty degeneration than do the three remaining cases. In this case fatty infiltration is also present and occurs principally on the periphery of the lobules and in the tract supplied by branches of the portal vein, while the fatty degeneration is more abundant in the central zone around the hepatic vein. In fatty degeneration the fat is usually in minute granules, which may coalesce to form small droplets, but only in the most advanced stages do they form large drops as is the case in fatty infiltration.

The sections in which the tissue has been treated with Fleming's solution show the lesion best, as the contrast of the black-stained fat is so marked as compared with the light hepatic parenchyma that photomicrographs may be readily made. The Soudan III sections, while valuable for demonstration purposes, can not be reproduced as well on account of less contrast and the red color.

The section in the case of rabbit No. 4 (Pl. III) shows a less advanced stage of fatty degeneration; there is also not much fatty infiltration present. In the section in the case of No. 2 (Pl. II) there is still less fatty degeneration present, while the section of rabbit No. 3 does not show any degeneration and only a little fatty infiltration.

## CONCLUSIONS.

The somewhat limited experimental data here reported point to the following conclusions, which may be altered by more extensive work on the subject.

### PRELIMINARY FEEDING PERIOD.

During the preliminary feeding period, the rabbits fed on organic phosphorus excreted a slightly larger proportion of nitrogen in the urine than did the rabbits fed on inorganic phosphates, but retained

[a] Made by B. J. Howard, Chief, Microchemical Laboratory, Bureau of Chemistry.

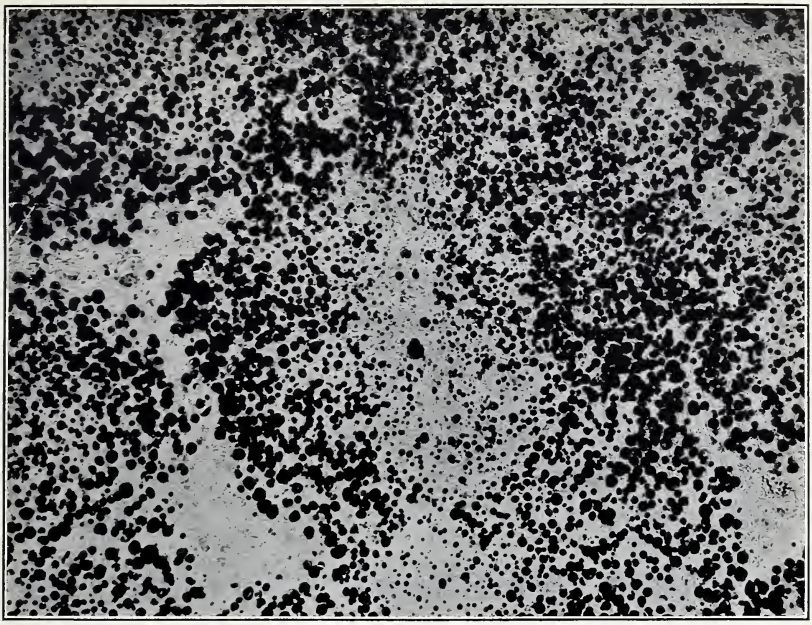

FIG. 1.—MAGNIFICATION 60 DIAMETERS.

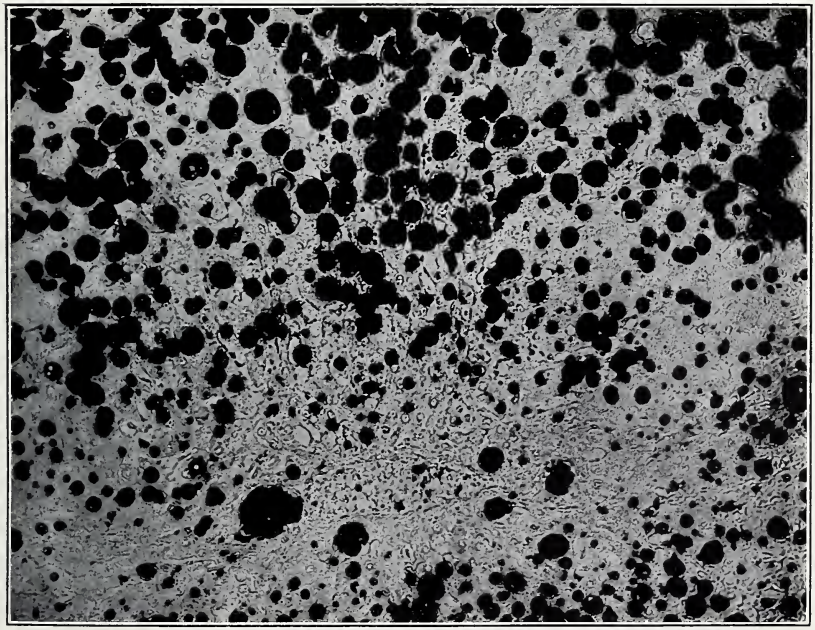

FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF ORGANIC-PHOSPHORUS-FED RABBIT NO. 1.

[Fat stained black with osmic acid.]

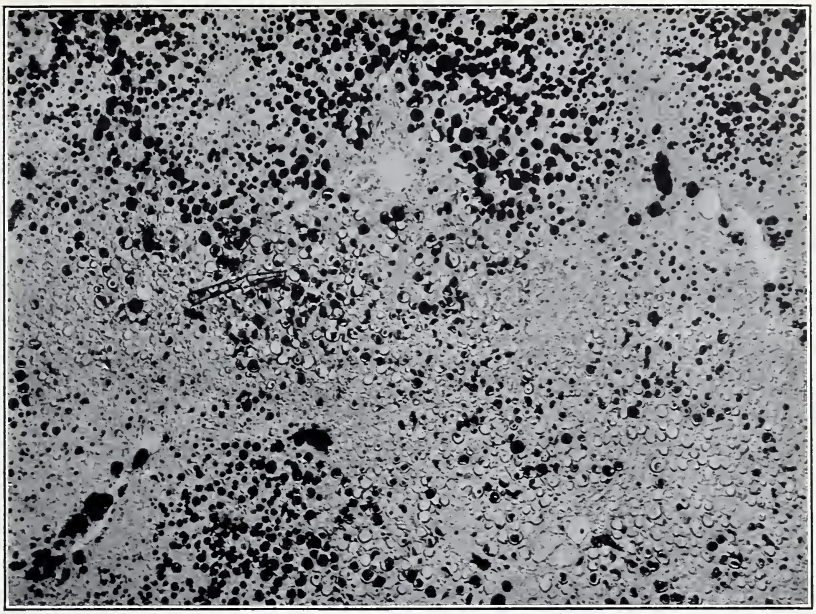

FIG. 1.—MAGNIFICATION 60 DIAMETERS.

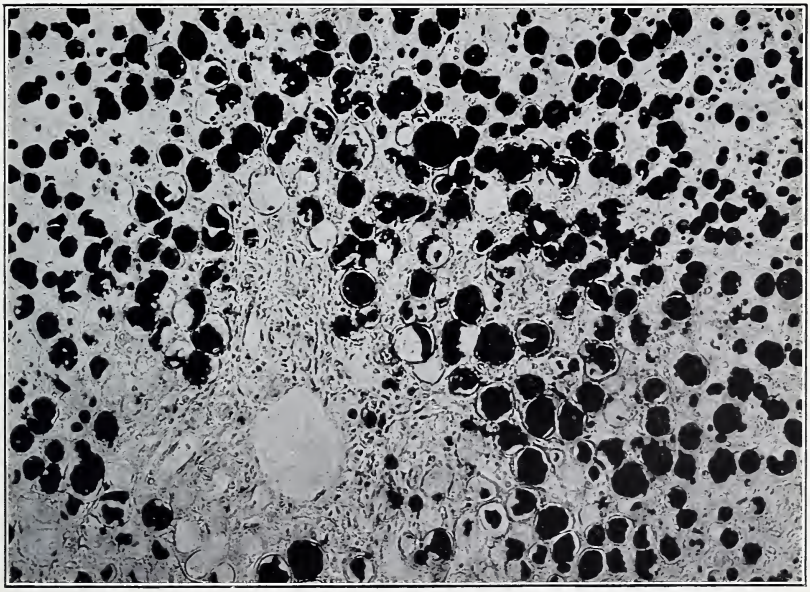

FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF ORGANIC-PHOSPHORUS-FED RABBIT NO. 2.

[Fat stained black with osmic acid.]

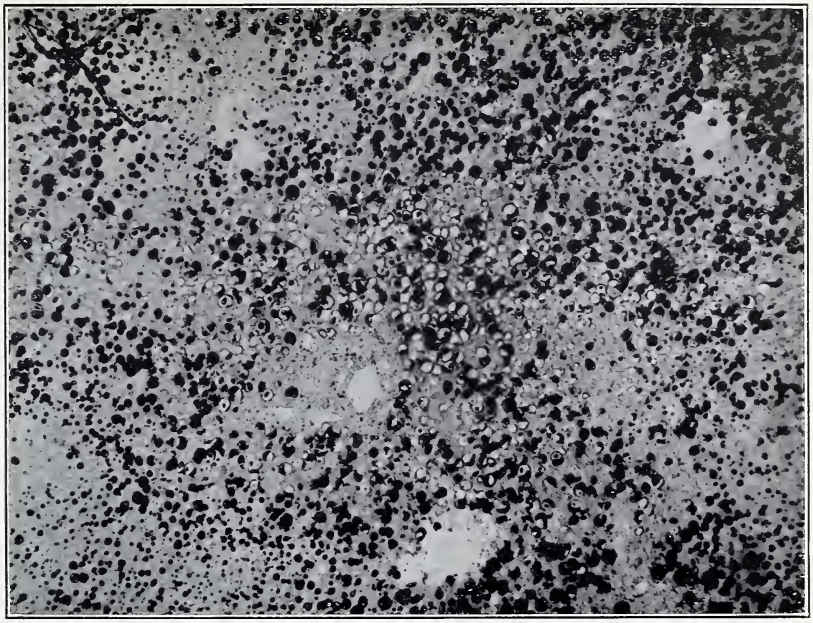

FIG. 1.—MAGNIFICATION 60 DIAMETERS.

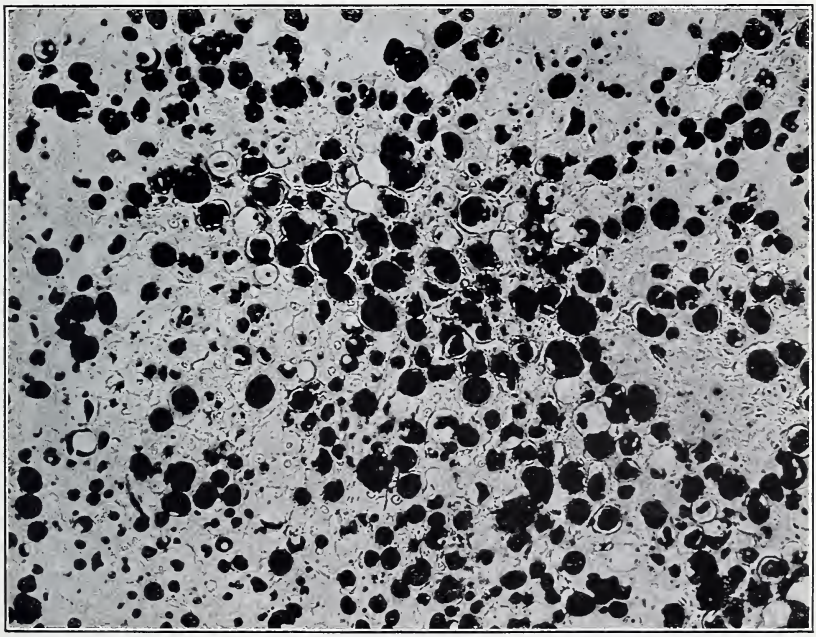

FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF INORGANIC-PHOSPHORUS-FED RABBIT NO. 4.

[Fat stained black with osmic acid.]

a smaller proportion of the absorbed nitrogen than did the rabbits fed inorganic phosphorus. With a few exceptions, the daily balances were positive.

The rabbits fed organic phosphorus excreted less phosphorus through the kidneys than did those fed inorganic phosphorus, but they retained a larger proportion of the absorbed phosphorus.

### PRINCIPAL FEEDING PERIOD.

The percentages of absorbed nitrogen retained in the case of the rabbits fed inorganic phosphorus show a wide variation, the average figure being practically the same as that for rabbit No. 1 fed organic phosphorus, rabbit No. 2 being excluded from the average. Accordingly, it is impossible to establish any effect produced on the nitrogen metabolism by the two forms of phosphorus fed.

A larger proportion of the ingested phosphorus was eliminated by the kidneys in the cases of the rabbits fed inorganic phosphorus than where the rabbits were fed organic phosphorus. The proportion of absorbed phosphorus retained is greater for the rabbits fed on organic phosphorus. The daily balances were positive with the exception of rabbit No. 2, which died two weeks before the close of the experiment.

The ether-alcohol-soluble phosphorus balances show that more phosphorus in this form was eliminated in the feces where phytin was fed than where inorganic phosphorus was fed.

No ether-alcohol-soluble phosphorus was found in the urine in any case, and it is doubtful if any organic phosphorus is present in the urine of a normal rabbit.

A smaller proportional amount of calcium was eliminated in the urine by the rabbits fed on inorganic phosphorus, but a larger proportional amount was retained in the body than in the cases of the rabbits fed on organic phosphorus. The same statement is true of magnesium.

### POST-MORTEM EXAMINATION.

A large proportion of the phosphorus of the fresh intestines of rabbits is dissolved by water during the process of cleaning preparatory to analysis.

The bones of rabbits fed on phosphorus for several months, either organic or inorganic, show a higher content of ether-soluble matter than do the bones of normal rabbits, and they form a larger percentage of the body weight than in the case of normal rabbits.

The livers of the rabbits fed on organic phosphorus for several months show fatty degeneration as well as fatty infiltration. Of the livers of the inorganic phosphorus fed rabbits No. 4 shows both fatty degeneration and fatty infiltration; in the case of No. 3, only slight fatty infiltration is shown. The livers are enlarged and contain

considerably more nitrogen and phosphoric acid than normal livers when calculated to a water and fat-free basis. In these experiments the degeneration and infiltration were most marked when organic phosphorus was ingested.

The brains and nerves of the rabbits fed on organic phosphorus yield a larger percentage of ether-alcohol soluble phosphorus than those of normal rabbits, while the brains and nerves of those fed inorganic phosphorus show lower figures. There is also a larger percentage content of ether-soluble material in the brains and nerves of the phosphorus-fed rabbits than is normal.

The tendency of organic phosphorus, especially in the form of phytin, to produce fat was strikingly indicated in this experiment as in the work of Jordan, Hart, and Patten. The autopsies showed that all the phosphorus-fed rabbits were in an abnormal condition, the livers being especially affected, and the histological examination confirmed the autopsy findings.

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